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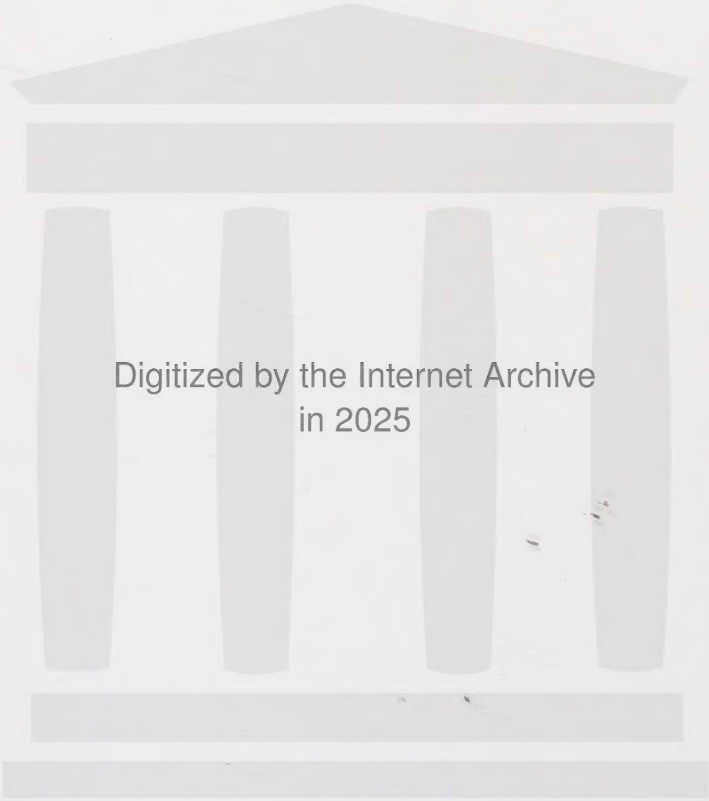
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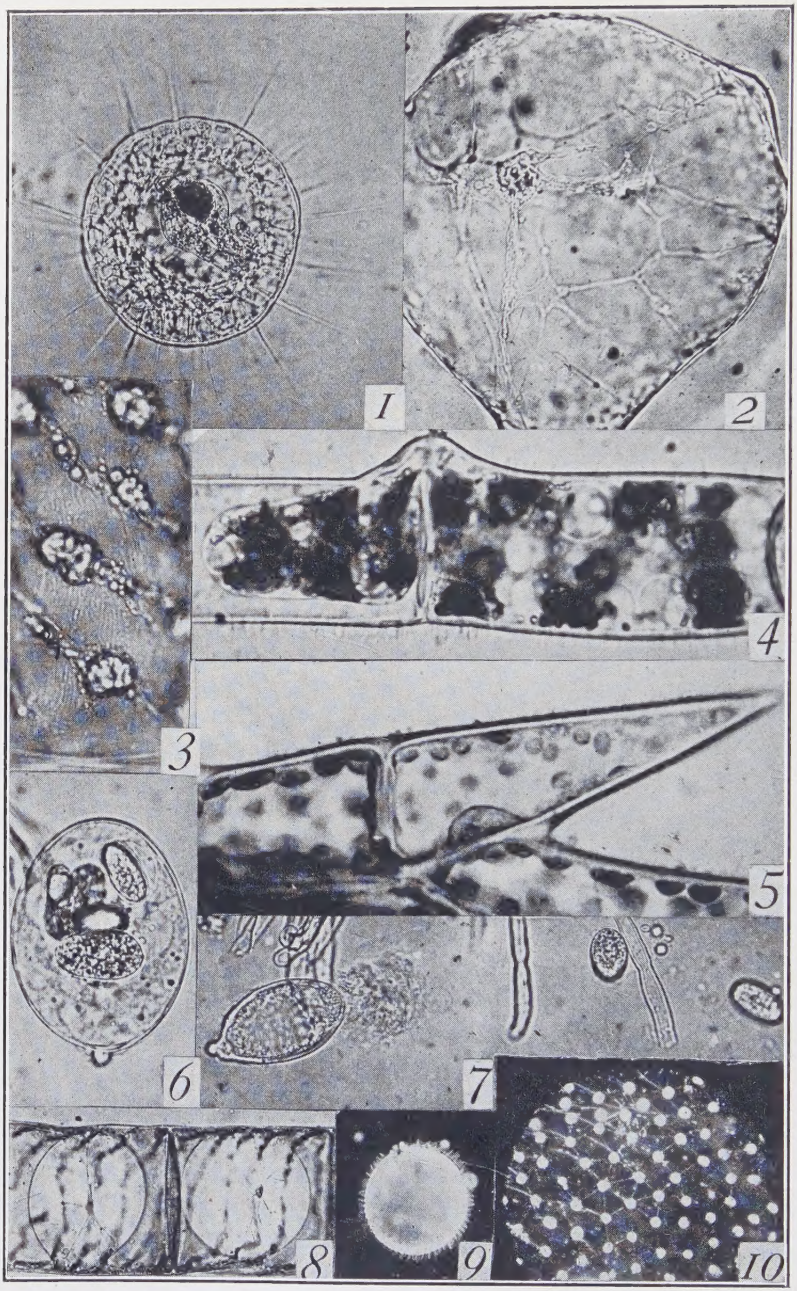
AN ELEMENTARY COURSE IN
GENERAL PHYSIOLOGY



Frontispiece

DESCRIPTION OF FRONTISPIECE

The figures illustrate certain features of cell form and function for which explanation is sought in the text.

(1) *Actinosphaerium*, a "sun animalcule," shows, for example, the extension of surface into filose pseudopodia used in locomotion; vacuolation of the protoplasm—the periodical bursting of the outer vacuoles gets rid of water which has entered the cell by osmosis; ingestion and digestion of food—a partially digested algal cell is seen lying in a large food vacuole.

(2) Cell from the fruit of *Symphoricarpus* (Snowberry), showing extension of the *inner* (vacuolar) surface of the cytoplasm as a complex network of strands which slowly change their shape and position.

(3) Portion of a cell of *Spirogyra nitida* focused on the cytoplasm to show fine streams of "kinoplasm" (with granules) apparently lying within the cytoplasmic layer and sometimes arising from the surface of the chloroplasts. The kinoplasm is in continuous and active movement.

(4) Conjugating *Spirogyra* cells. The "male" gamete (left) is passing by a species of amoeboid movement through a narrow connecting tube in the papilla (above) into the "female" cell, where fusion takes place. Both gametes show abundant contractile vacuoles, by the discharge of which the cells get rid of water and reduce volume. The water comes from the large sap vacuole, which finally in the zygospore entirely disappears. Here we have an example of typical plant cells becoming animal-like in their behaviour during the phase of reproduction.

(5) Marginal cells of the leaf of *Elodea* showing the characteristic structure of mature plant cells. Nucleus and chloroplasts lie close to the walls, held in a scarcely visible film of cytoplasm. They are flattened by the pressure of the sap vacuole which occupies the bulk of the cell cavity. Streams of kinoplasm commonly carry the chloroplasts around the cell in a movement of "rotation" or "cyclosis."

(6) Ascus of *Uncinula* (a mildew fungus) soon after its release from the dry fruit body into water. It shows four spores lying in vacuolated protoplasm. The vacuoles arise as a result of absorption of water. In this case they are not contractile, as in (1) and (4), but retain their water, causing the ascus to swell until its wall ruptures and the contents are explosively ejected.

(7) The same ascus (less magnified) after the discharge of its spores, two of which are seen at the right, the others being projected much farther. Some of the protoplasmic content of the ascus is also thrown out. Although most of this is seen to mingle diffusely with the medium, one or two intact vacuoles are dimly visible at the limit of the mass.

(8) Semipermeability of the vacuolar membrane *per se* in *Spirogyra* cells some hours after treatment with iodine, eosin and glycerine. The vacuolar membrane has plasmolyzed independently although the cytoplasm and nucleus are stained and apparently dead. Outside the vacuolar membrane is eosin solution, inside is little or none, as shown by the transparency of the vacuole. The membrane has a smooth minimal area contour indicating that it has not coagulated like the rest of the cell.

(9) Young colony of *Volvox* under dark field illumination, showing cilia.

(10) *Volvox* under dark field illumination, showing the protoplasmic connections (plasmodesmae) between the cells.

AN ELEMENTARY COURSE IN
GENERAL PHYSIOLOGY

PART I—PRINCIPLES AND THEORY

BY

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PART II—LABORATORY EXERCISES

BY

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PREFACE

The following course is designed as an introduction to the study of physiological mechanism equally in plant and animal life; that is, to the analysis in terms of physical and colloidal chemistry of the general properties and behaviour of cells. Such a study, commonly termed General Physiology, is admittedly an important phase in the education of present-day students of biology and medicine, but it is handicapped by a lack of elementary textbooks, especially such as might be acceptable to plant physiologists. The expressions of appreciation which have come from many teachers into whose hands has fallen a small pamphlet of Laboratory Exercises, printed some years ago for our own use, has induced us to elaborate the course there presented and offer it to a wider circle; while the oft-repeated request of our own students for a theoretical textbook on the subject has led one of us to attempt supplying that need also.

Since it is necessary to stress the fundamentals, our plan is first to drive home those physical facts and principles which are most useful in physiology, and second to indicate some of the many possible applications of these to the life of the cell. At the same time the student is guided to an appreciation of the complex organization of cells and of living matter, so that in becoming a physical chemist he will not cease to be a biologist.

One of our guiding principles is to base instruction so far as possible on individual work. With large classes this means simple experiments and inexpensive apparatus. There are a few departures from this rule, mainly in the use of the potentiometer, the technical importance of which requires its inclusion. But these more elaborate experiments are intended rather for smaller groups who are specializing in physiology.

So far as convenient it is sought also to build up an historical background by the repetition of classical experiments. To know that he is repeating the experience of a Bütschli or a De Vries may cause one to feel that blowing soap bubbles, for examples, is not

infra dig, even for a college student. It is a mistake to suppose that this is a fit occupation only for children and professors!

As to the division of responsibility between the authors, it is enough to state that the inception of the course and the individuality of much of the practical work are due to the senior author, while the junior is responsible for Part I of the book, for sections of Part II and for the literary work in general. The drawings were kindly executed by our colleague, Mr. R. D. Gibbs.

We express our thanks to those authors and publishers from whom are borrowed illustrations and experiments. Our debt to the works of reference named hereafter extends far beyond the more direct borrowing, for which formal permission was requested, to the reception of ideas and inspiration which we gratefully acknowledge.

F. E. Lloyd,
G. W. Scarth.

NOTE TO INSTRUCTORS

The theoretical section of this book is not put forward as a comprehensive students' textbook. The reference to many subjects is frankly suggestive rather than exhaustive and is meant to be supplemented by the teacher or by wider reading on the part of the student. For this purpose there is given at the end of Part I a list of readings and references for each chapter. These include textbooks, reviews and monographs in which fuller treatment of the various topics may be found. An extensive bibliography of original papers, however, would be superfluous since such lists are to be found in the works referred to. Occasionally where a subject of recent investigation receives summary reference in the text, a footnote is added as to the source.

For elementary students certain sections such as those dealing with electric potential and the more elaborate experiments may well be omitted. To facilitate shortening of the Laboratory Course, if so desired, the more elaborate and difficult experiments are marked with an asterisk, whereas other exercises which may be left out in reducing the course to the space of half a term are enclosed in brackets.

Full lists of the equipment, material and chemicals required will be found in the Appendix.

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ACKNOWLEDGMENT OF TEXT FIGURES

FIG. 1*a*.—Taken by permission from W. Pfeffer's "Physiology of Plants," Trans. and Ed. by A. J. Ewart, published by the Clarendon Press.

FIG. 1*b*.—Taken by permission from Robert Chambers in E. V. Cowdry's "General Cytology," published by the University of Chicago Press.

FIGS. 3 and 5.—Adapted by permission from E. V. Cowdry in "General Cytology," published by the University of Chicago Press.

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FIG. 36.—Adapted by permission from W. M. Clark's "The Determination of Hydrogen Ions," published by the Williams & Wilkins Company, Baltimore.

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FIG. 65.—Taken by permission from W. M. Clark's "The Determination of Hydrogen Ions," published by the Williams & Wilkins Company, Baltimore.

AN ELEMENTARY COURSE IN GENERAL PHYSIOLOGY

CHAPTER I

LIFE AS A MECHANISM

In contemplating vital processes we are at once confronted with phenomena which appear to be peculiar to life and require terms that are not used in physical science, for example, respiration, assimilation, excretion, reaction to stimulus, adaptation, reproduction, etc. But the increasing tendency of modern physiology is to analyze these into more fundamental, that is, physical and chemical, mechanism. General Physiology, including Cellular Physiology, is that branch of the science which confines itself entirely to this ultimate stage of analysis.

1. Mechanism v. Vitalism.—In connection with what has been said, the question arises whether the treatment of life processes as purely physical and chemical does not involve a fallacy. Are we justified in studying protoplasm as if it were merely a rather complex physical substance? To do so requires an act of faith just as much as does taking up the view that it defies such treatment. On the whole the success which has rewarded the investigation of mechanism by physical methods is its best justification. On the other hand, as the light spreads so does our appreciation of the abyss of darkness that stretches beyond. Hence it is possible that there will always be two opposing opinions (determined by viewpoint or by temperament) holding respectively the doctrines of *mechanism* and of *vitalism*.

It is interesting to note historically how the battleground on this issue has shifted its position. The ancients were essentially vitalists in that they assumed a distinctive element in life, although opinion as to its nature hovered between an immaterial soul

(psyche, anima) and an excessively subtle material substance, the **pneuma** of Hippocrates and Galen, the **spiritus** of Fernelius. When, inspired by Harvey, experimental physiology revived in the 17th century, a rather narrow mechanistic view of life became fashionable among groups of physiologists (the so-called **iatrophysical** and **iatrochemical** schools) and it was as a reaction to this that the doctrine of vitalism and of a vital force first received formal and vigorous expression.

Man has always felt subjectively that he has within him a source of power which can control and exercise force upon his physical body, and when the experiments of Haller (seventeenth century) showed that excised muscles retain for a time their unexplained irritability or responsiveness to stimulation, the doctrine arose that every part of a living body is imbued with a special *vital force*. All life phenomena which were not understood came to be ascribed to this mystical agency. The whole tendency of later research, however, has been to push back the boundary of the purely vital.

The long reigning, half mystical doctrine of " animal spirits " (Galen) was replaced when Lavoisier showed that animals breathe *oxygen*, and that respiration is akin to combustion.

A strong citadel of vitalism, namely, the existence of a set of substances, the carbon compounds, which are produced in nature only by organisms (therefore called organic) and which it was believed were not producible apart from life, was first breached by Wohler's synthesis of urea from inorganic elements. The conquest has proceeded apace ever since and there is no apparent reason why it should halt.

Finally, with regard to a " vital force," as methods of estimating energy developed and as the energy balance of organisms was determined first by Mayer and later in a long series of increasingly refined calorimetric measurements, the lack of any extraneous (psychic) source of energy became more and more certain. For example, during muscular work in man the chemical energy spent (measured by oxidation) was found to balance the energy developed in mechanical work, bodily processes and heat to within a fraction of one per cent (Atwater and Benedict).[1] The principle of the conservation of energy is now one of the pillars of physiological as it is of physical science.

[1] See, for example, Mitchell, P. H.; "General Physiology" Chapter XXIV.

The doctrine of vitalism has therefore had to shift its ground once more. Some modern vitalists (neo-vitalists) hold that without transgressing the law of conservation a special form of energy may be produced in organisms from the chemical energy of their food. But this is a pure assumption which helps us to understand nothing. Others ascribe the peculiar features of life, especially those of its organization, to an unknown psychic element (entelechy, etc.) which " directs " the physical and chemical forces. Until it can be shown, however, that cause and effect act differently in animate and inanimate nature, that some vital process is contrary to the laws of physics and chemistry, the application of these laws to life is justified. In point of fact, no specific instance of such subversion of physical law, for example those cited by Driesch with reference to the development of ova (see Wilson, The Cell, p. 1116), has yet stood the test of fuller knowledge.

2. Indefinability of Life.—The needlessness of postulating a purely vital element is further shown by the lack of any distinctive criterion of life and the impossibility of deciding in many instances whether bodies are alive or not. As to criteria of life the characters we associate with living matter can all be duplicated—crudely, but for all we know, essentially—in behaviours of lifeless matter. Take a few of the most characteristic:

(a) *Unpredictable Movement* is neither essential nor peculiar to life. " Who can predict the orbit of a fly?" says the vitalist. But who also can predict the orbit of a single particle in Brownian movement? Statistically the movement of particles and of molecules is predictable, but conceivably the same may be said of flies. With a single particle the factors are so complex as to appear free from law. Still more complex are the factors which determine movement in living matter.

(b) *Growth by Assimilation of a Heterologous Medium.* In the growth of organisms new stuff is elaborated from simpler elements and deposited between or upon already formed particles. These features are duplicated essentially, in for example, the growth of a " cobalt tree " and other forms that mimic life, as will be seen later.

(c) *Reproduction and Persistence of the Type* is accomplished in the simplest case by separation through a rearrangement of forces, internal and external, of a part which can grow until it

resembles the parent body. A crude physical analogy is that of a snowball rolling down a long hill and throwing off fragments which repeat its own history of growth and subdivision.

(d) *Adaptation* is the property conspicuously possessed by living beings of changing, within certain limits, their character with changing conditions, in such a way that they may continue to exist. Such a property, however, is essential to the persistence of any unstable unit in a mutable environment, and, given the property of change—which is common to all nature, the frequency of adaptive modifications in living organisms is explained, according to the Darwinian theory, by the survival of only those types that happen to possess them.

(e) *Organization*, applied to life, means the unifying principle in vital processes or the integration of the many parts of a living being into a single " whole " which exhibits an individuality of its own. The present tendency in philosophy is to extend the conception of organization as a fundamental feature of existence from the realm of biology to the universe in general. Some elaboration of this point is necessary.

3. Vital Organization.—The very name by which living beings are designated, namely, " organism," is eloquent of the fact that they possess in a high degree that elusive quality which we call organization. The term, as we have said, is sometimes applied to inanimate objects, from astronomical systems down to atoms. It implies that the parts of the object are differentiated and exhibit an orderly arrangement and relation to one another and that the whole forms a more or less complete entity or individual—in short, differentiation, ordered arrangement and integration. The question is whether the underlying cause of the phenomenon is the same in living as in lifeless matter.

The mystery and complexity of its organization is the most staggering feature of life. How the assemblage of atoms in a human body comes to act as a man must forever remain beyond the grasp of the mind of man. But if we do not attempt to envisage the whole melée and are content to analyze it, we find that the vital merges into the purely physical. Man is made up of smaller units, namely, cells, which are independently alive and capable of remaining so apart from the body. We find again that the cell, which is still an organization of inconceivable complexity, appears to be made up partly of yet smaller living units which

reproduce themselves and which appear to find their counterpart in similar self-propagating (?) units which are not organized as cells, viz.: the so-called filterable viruses, known chiefly by their injurious effects on larger organisms. These latter units are so much more akin to enzymes—lifeless colloidal particles—than to cells that there is still doubt as to their vitality.

Thus it is uncertain at what stage of complexity *life* begins. But wherever the line be drawn it is clear that *organization* as above defined does not begin here. It extends downward to molecules, atoms and electrons. The ultimate units of all matter—or energy—so far as we know at present, are protons and electrons—positive and negative electric charges. Whether any new factor enters into the organization when the stage of life is reached or whether there is only a difference of degree is a philosophical question that cannot be answered at present. The continuity of the series outlined above, the lack of any sharp break, or indeed of any certain dividing line between living and non-living rather points to a fundamental unity. It appears therefore that the only indubitable distinction of vital organization is its superior complexity, and if the physical analogues of vital characteristics quoted above appear crude and superficial, the excuse is that they cannot be otherwise on account of the immeasurable difference in complexity between non-living and the higher forms of living organization.

From the point of view of the physiologist life may be analyzed as a complex, highly organized set of energy transformations. From the standpoint of a modern school of philosophy the whole may nevertheless be something more than its parts, just as the properties of NaCl appear not to be revealed in those of Na and Cl taken separately. New relationships bring new properties. Life, it is said, may "emerge" as a "new" thing when a certain stage of complexity of physical organization is attained. Similarly higher forms of life appear with further combinations. Observation tells us that even *mind* is connected through all gradations with mindless existence, and may be regarded as having somewhat the same relation to life as life has to lifeless energy.

Since the same fundamental physical laws apply to the most complex as to the simplest chemical compounds, there is no reason to imagine that their validity will fail in still higher—vital—degrees of complexity. Experience indeed has amply justified the

application of physical law to vital phenomena but in any case there is no alternative because there is no other scientific avenue of approach.

In relating our faith in mechanism as a scientific doctrine to a philosophy of life, it is well to remember, however: (1) that the analysis of mechanism, since it breaks up relationships and sacrifices organization, cannot fully explain life any more than a sonata is explained by the notes that compose it; and (2) that even an analytical explanation can never be final since the fundamentals of physics are ever receding.

CHAPTER II

ORGANIZATION OF PROTOPLASM—CELL STRUCTURE

The general information regarding cell structure embodied in this chapter is intended to serve mainly as a background to intensive study of the particular forms used for experiment. Close familiarity with one's material rather than a general and theoretical knowledge of cytology is the essential thing in experiments with cells.

1. Types of Vital Organization.—Protoplasm, " the physical basis of life " as Huxley termed it, is a stuff which everywhere has similar properties. It is more than a stuff, however; it is also an organized mechanism. What the least organization compatible with vitality is we do not know. It can at least exist on a very small scale. Filter-passing organisms—allowing them the right to the title—are no larger than the finest colloidal particles. Not a very large number of molecules even may enter into their composition. It would seem that the superior complexity of organization as compared with a lifeless particle must be mainly in the way these molecules are arranged and orientated, but any further hypotheses on the subject must wait till we have studied the properties of colloid particles.

In ordinary bacteria, ranging perhaps 100 times the diameter and a million times the volume of the " filter passers," there is room for immeasurably more organization, but the scale is still too small to distinguish much structure. A membrane on the outside and a few granules inside are all that can be seen. In common with the condition in many of their allies, the blue-green algae, a definite nucleus is indistinguishable—though the granules may be nuclear material. In all higher forms the living substance is always nucleated. It is differentiated into at least two portions, **nucleus** and **cytoplasm.**

If we start with the organism as a whole as our unit, we find that its development consists in the growth and differentiation of

protoplasm and of materials produced by the protoplasm. The modes of development vary greatly. Among the Protista a high degree of differentiation occurs within a body which contains only one nucleus. In such cases the size is always small. Coenocytic forms which consist of multinucleate masses of protoplasm attain much larger size but not much greater differentiation. The highest differentiation is associated with cell formation. Frequently, however, there is a coenocytic stage in the development of tissue which later becomes cellular. It seems better, therefore, to regard cells as a differentiation of the organism than the organism as an aggregate of cells. When protoplasm is separated into cells either by a cell wall as in plants or by an " intercellular cement " as in some animal tissues, connecting strands of protoplasm (plasmodesmae) are sometimes recognizable. More commonly they cannot be detected. Although invisible connections are possible it is not necessary to assume their existence since the passage of electrical currents and of diffusible substances may explain the influence of one part on another.

Although the cell is not an invariable unit in the differentiation of protoplasm, it is a very important one in that it is the smallest subdivision of a larger living body which is capable of independent existence. Even in the most individualistic organisms, that is, the higher animals, where the parts are so interdependent that a tap on the head for example may speedily result in the disintegration of the whole protoplasmic system, the cells are capable of independent existence apart from the body if a suitable environment is provided, as shown by their indefinite growth in tissue cultures. The distinct parts of a cell, on the contrary, cannot continue to live except in combination with the rest. The irreducible organization of protoplasm as we generally know it is therefore the minimum organization of a nucleated cell.

2. Structure of the Cell. (*a*) *Essential Structures.*—At its very simplest a cell may show no visible structural differentiation except into nucleus and cytoplasm, both optically empty. We may here diverge for a moment to discuss the obvious properties of protoplasm as a " stuff." We find that in its simplest form both in cytoplasm and nucleus it appears *clear*, *colourless*, and *structureless*, under even the highest powers of the microscope; that it is generally a rather viscid liquid though in parts it may be quite fluid and in others of jellylike consistency; and that it does

not mix with water. In view of the fact that it is largely composed of water (65 to 90 per cent when active) this immiscibility is notable and there are two theories to account for it. One regards protoplasm as an organic liquid insoluble in water but acting as a solvent for water (Fischer, Lepeschkin, etc.). The other view is that there is a film of water-immiscible substance in the exposed surface of protoplasm but that the bulk of it is a colloidal solution *in* water. When the protoplasm is spilled out of a plant cell into a watery medium, it either partly disperses in the medium and partly coagulates into an amorphous clot, or else droplets are formed which remain discrete from the medium and apparently alive (Fig. 1*a*). A similar alternative occurs when

Fig. 1*a*.

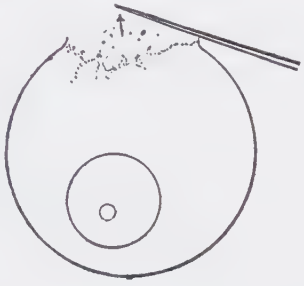

Fig. 1*b*.

Fig. 1*a*.—(Pfeffer.) Tip of root hair of Frogbit (*Hydrocharis morsusranae*) burst open at top under pressure of coverglass. The protoplasm, oozing out, forms globules. Note also the vacuoles which enlarge by absorbing water.

Fig. 1*b*.—Diagram of starfish egg. The surface is torn suddenly with the result that the protoplasm disperses in the water.

Taken by permission from Robert Chambers in E. V. Cowdry's "General Cytology," published by the University of Chicago Press.

the inner fluid protoplasm of, for example, an amoeba or a starfish egg, escapes through a tear in the stiffer cortex. The surface film here is liquid but it is not merely the boundary between two immiscible liquids as its behaviour under micro-dissection shows. It is rather like the film of a soap bubble in that it withstands gentle manipulation but disintegrates under violent treatment, with the result that the cytoplasmic content merges in the water and its granules scatter and disappear (Fig. 1*b*). It has also been found that dyes such as eosin, soluble in water, insoluble in

fats, and unable to enter cells appreciably from the outside, nevertheless diffuse freely throughout the protoplasm when injected by a fine pipette beneath the surface layer. We must conclude therefore, for such cells at least, that the bulk of the protoplasm being permeable to eosin and miscible in water is different from the superficial layer, and has water as its solvent or continuous medium.

To the layer, fluid though it may be, which thus acts as a barrier between the more aqueous protoplasm and the water outside, the name **plasma membrane** is applied. Though definitely proved in some cases, the universality of a " plasma membrane " or film is hypothetical, but, if we accept it, the differentiation must be counted as a third essential structure in addition to nucleus and undifferentiated cytoplasm. For the immediate life of the cell it is even more essential than the nucleus, because cells will live for a long time without a nucleus (amoeba, two weeks, human erythrocyte, three weeks) whereas death is immediate unless a break in the plasma membrane is repaired.

Similar to the plasma membrane proper are the films which exist at the internal surfaces of the cytoplasm where contact is made with the nucleus, vacuoles, plastids, and other inclusions, and close observation further reveals that there is usually within the body of the cytoplasm a commonly active unstable system of processes, streams, fibrils or films, sharply defined from the matrix but apparently continuous with and similar in character to the surface films. Similar processes may extend externally from the plasma membrane or into the vacuole from its membrane. It is convenient to group all these varied but similar structures under the term *kinoplasm* (Strasburger) as distinct from the cytoplasmic matrix (less aptly termed *trophoplasm*). See Fig. 2.

The **nucleus** has a membrane of its own in contact with the enveloping membrane which belongs to the cytoplasm. The nuclear interior is sometimes fluid and perfectly homogeneous not only to the eye but apparently also to the needle (Chambers), although in plants there is some evidence of a fine structural network even in optically homogeneous nuclei. In other cases, especially in plants, there is a definite gelatinous framework of a more massive character. Even in the case of homogeneous nuclei, however, the chromosomes which appear only at mitosis have a genetic continuity which is hard to understand without assuming

some persistent structure. A nucleolus or nucleoli are commonly present.

(*b*) *Other Common Visible Structures.*—**Plastids**, including chloro-, leuco- and chromoplasts, are common in plant cells. They are definitely protoplasmic structures of varied form, active in the synthesis of carbohydrate. They are of more gelatinous consistency, at least at their periphery, than the cytoplasmic matrix.

The visible appearance of the cytoplasm is much more frequently heterogeneous than homogeneous. **Granules** of various kinds are very common. Among the easiest to recognize specifi-

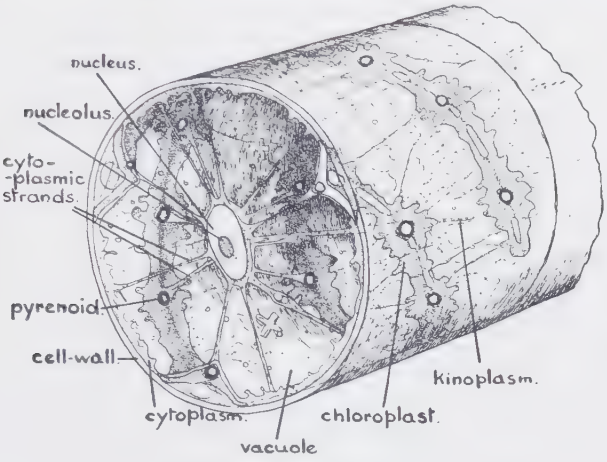

Fig. 2.—Diagram of a *Spirogyra* cell cut across.

cally are the **mitochondria** (also called chondriosomes), particles rich in lecithin, which strongly absorb many vital (basic) stains and reduce Janus Green B from greenish blue to pink and colourless. Granules are mainly located in or adhering to the kinoplasm, when the latter is abundantly present. Food granules are also common (see below). The above structures are more solid than the cytoplasmic matrix but more liquid regions (alveoli) are also abundant in some kinds of protoplasm. The commonest variety may be regarded as a separation of a watery phase of the cytoplasm, eventually becoming typical **vacuoles** as dilution proceeds. The name **vacuome** is given to structures which are stages in the development of vacuoles proper. Sometimes the elements of the vacuome are spherical, sometimes drawn out into irregular tubules,

as may be seen in living as well as fixed material (Fig. 3). In mature plant cells the large single vacuole occupies the greater part of the cell (Fig. 4). The so-called **Golgi apparatus** which is detected in many animal cells (Fig. 5) seems to represent an irregularly shaped vacuome structure. A vacuole consists of sap and an enveloping membrane. The sap often stains deeply in life with basic dyes such as neutral red.

Mention may also be made of the extra-protoplasmic **cell wall** of plants and the **intercellular substance** of certain animal tissues.

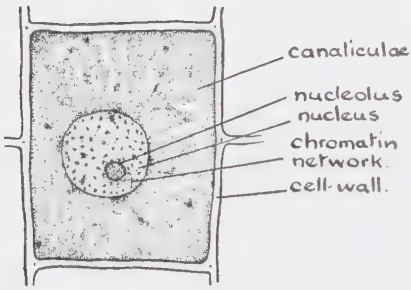

Fig. 3.

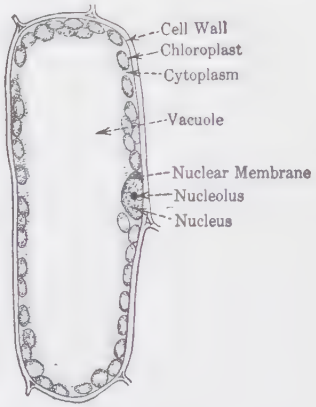

Fig. 4.

Fig. 3.—Young plant cell from root tip of Onion, fixed and stained. The clear canaliculae may represent the vacuome (Cf. Fig. 2*b*).

Adapted by permission from E. V. Cowdry in "General Cytology," published by the University of Chicago Press.

Fig. 4.—(Holman and Robbins.) Mature plant cell from a leaf.

More specialized yet common are **fibrillar structures**, such as contractile fibrils, conducting (nerve) fibers, cilia and flagella which may be developed from kinoplasm but are doubtfully protoplasmic.

Finally protoplasm may include bodies which in themselves are totally inactive, for example, food bodies (starch, proteins, fats, etc.), mineral crystals, pigments, etc.

(*c*) *Colloidal Structure of Protoplasm.*—In addition to that which is visible there is structure too fine for ordinary microscopic perception but to some extent revealed in other ways. Microdissection, for example, shows that mobile plastic protoplasm

may be elastic almost like a jelly, which implies some kind of solid structure. Localization of substances and reactions points to the same conclusion. At the dimensions of colloid particles, structural properties merge into the physico-chemical or substantial properties, and will receive further attention later.

3. Aids to the Microscopic Study of Cell Structure.—The bounds to our perception of structure by ordinary microscopic observation are imposed not only by a definite minimum size limit of visibility determined by the wave length of light, but also sometimes by lack of optical heterogeneity to correspond with any physical heterogeneity which may be present. Thus glass beads are invisible in Canada balsam because they have the same

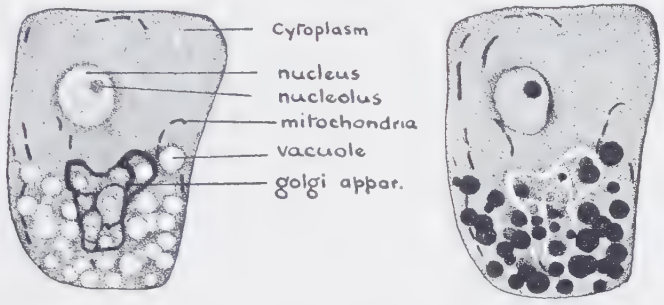

Fig. 5.—(After Cowdry.) (*a*) Animal gland cell (from pancreas of guinea pig) blackened with osmic acid to show fatty elements. (*b*) The same cell bleached and stained with iron haematoxylin. The Golgi apparatus shows as a clear network.

Adapted by permission from E. V. Cowdry in "General Cytology," published by the University of Chicago Press.

refractive index. Coloured glass, however, is visible; and on the same principle cytologists have long resorted to *post mortem* **staining** as an aid to the observation of cell structure. Also by impregnating the cells with Canada balsam, they are able to use effectively oil immersion objectives of wide aperture bringing the utmost magnification and resolution to bear on minute objects. In order to preserve as far as possible their normal architecture after death, the cells are "fixed," as it is called, by agents which quickly penetrate, kill and coagulate the protoplasm. Experiment shows, however—and our study of colloids will explain why—that the finer structure as it appears in a cell so treated varies with the fixing agent and can have but little relation to the

natural state. The brightly hued slides of post mortem histology have been dubbed "painted tombstones." Regarding the living characters of the deceased, they are as veracious as most epitaphs. This is perhaps rather an ungenerous description of a method that has contributed to science our present knowledge of chromosomes, for example; but chromosomes are relatively large structures and are visible in life. The point is that the suspicion of artefact (artificial production) must always attach to structures which cannot be detected in normal healthy cells. Certain cell structures can be stained during life, but although "**vital staining**" has its uses, as shown in a later section, it is limited in its application and probably no structures are revealed by its aid that cannot be seen without it.

Another method of discovering physical differentiation in cells is the use of **ultra-violet photography.** Structures or substances differ to a much greater degree in their transparency to ultra-violet than to visible light and may be distinguished in photographs taken by this kind of illumination. The chromatin of the nucleus is particularly opaque under these conditions. The nature of the apparatus required, including quartz lenses throughout, has restricted the use of this technique in the past. Its future success depends on the extent to which the cells may prove to be affected by the exposure.

The uses and results of **dark-field microscopy** (ultra-microscopy) are explained in dealing with the colloidal structure of protoplasm, with respect to which the method is mainly useful.

Quite another principle is involved in the exploration for structure by means of **micrurgy**—or micro-dissection, by probing cells with extremely fine glass needles mechanically controlled. The method has helped to establish the existence of protoplasmic membranes on and in the cell, as well as other structures; but its main use has been to increase our knowledge of the physical properties of different parts of the cell -properties which depend upon colloidal structure, later dealt with.

4. Living and Non-Living Elements.—If parts of an organism may be non-living, as undoubtedly they may, there is no reason for denying the possibility that parts of a cell may be non-living also. It is indeed difficult to regard a starch grain or a crystal as alive simply because it happens to lie within a living cell. It is impossible, however, to draw the line between what is alive and

what is not. There are all grades of activity and lability. If we adopt as a criterion of life that a living structure alters with the death of the cell whereas a non-living one does not, then only such stable inclusions as food bodies and excreta are dead but mitochondria and other granules and even the vacuolar sap and cell wall are alive. These and other structures which are less active than the protoplasm proper but may yet be regarded as alive are sometimes distinguished as **metaplasm** or **alloplasm,** and dead substances as **ergastic material.**

The fact that certain elements of the cell are self-propagating has led to another hypothesis, that self-reproduction is the distinctive feature of all living intracellular as well as larger units. We know little, however, as to the extent of this character. Facts of heredity and cytology point to the existence of autonomous units—the so-called genes—within the nucleus. In the cytoplasm, plastids (in plants) multiply, sometimes at least, by division, and so may mitochondria and other structures. But none of these cytoplasmic bodies are universally present in protoplasm. Also they appear sometimes to be produced *de novo*. It is suggested, however, that the material of which they are composed may be self-propagative even if the visible bodies disappear and reappear, just as the hypothetical genes are presumed to persist although the chromosomes of which they are elements have no continuous existence. Those who ascribe all transmission of hereditary character to the genes would picture the cell as a kind of house which these living units build for themselves. There is evidence, however, that the specificity of cells depends also on the cytoplasmic material allotted to them in cell division, particularly on the hyaline ground substance. That being so, a theory of self-propagating units to be in accord with the facts must regard the cell rather as consisting mainly of a colony of ultramicroscopic units ("bionts") bound together in obligative symbiosis. These "bionts" are compared to the filtrable viruses and bacteriophage which do not combine as cells but which are obligative parasites.

In contradistinction to such hypotheses is the point of view which stresses organization as the essential features of life. It regards *all* the elements of protoplasm as non-living and only the system as living. But the same question arises, what system? Whatever system you choose, a certain environment physical as well as organic is essential for its existence.

5. Chemical Composition of Protoplasm.—The chemical composition is imperfectly known partly because of its complexity and partly because the stuff ceases to be protoplasm when it is analyzed. As previously mentioned water forms 65 to 90 per cent by weight of active protoplasm, although it may be reduced to 10 per cent or less in the dormant state. The other analytical ingredients which are known to be essential are proteins, lipoids and inorganic salts.

The bulk of the dry substance of protoplasm is protein. The possible number of varieties of proteins is inconceivably great and protein diversity is probably one factor at least determining specific differences. It is illustrated in the specificity of reaction to protein poisoning and the distinct nature of hemoglobins in different species. Chemically we are probably dealing with protoplasms rather than protoplasm. Much of the protein is combined (conjugated), especially with nucleic acid to form nucleo-proteins. These, as their name indicates, are particularly characteristic of the nucleus, representing what is optically distinguished as "chromatin," the substance of which the chromosomes mainly consist. According to many, the proteins are also combined with lipoids. This is not so certain, although some kind of union—by adsorption (see later) if not truly chemical—is most likely. Combinations of proteins with carbohydrates and with one another are also believed to exist.

Of the **fatty compounds** the ordinary fats vary in amount with the state of nutrition and are probably reserve food. On the other hand "lipoids" (fat-like bodies not decomposed by alkali), though forming only a small fraction of the dry weight, are probably as essential as the proteins. In the case of animals at least, no matter how starved the tissue, the lipoids never fall below a certain proportion. Of the lipoids two which are said to be common in animal cells, namely, lecithin (a phospholipin) and cholesterol (a complex alcohol), have had important functions ascribed to them. It is doubtful at present if lecithin or other phospholipin is an element of plant protoplasm, although phosphorus-containing compounds of somewhat similar physical characters are present (a Ca salt of a diglyceride-phosphoric acid—Chibnall and Channon, Biochem. J., **21**, 1927). Also in plants cholesterol is replaced by phytosterol. The important fact, however, is that a certain proportion of substance, which, whatever its exact chemical nature,

has certain peculiar properties characteristic of lipoids, is probably always present.

The carbohydrates unless combined with proteins are not known to be an essential element of the protoplasm itself although they play various important rôles in nutrition, protection, etc. Inorganic salts though small in amount do play an essential part especially in maintaining the organic material of the cell in a proper physical state, as we shall see later.

The proportion of the various analytical components of protoplasm varies in different parts of a cell. Nucleic acid and nucleoprotein are found mainly in the nucleus. If there are really self-propagating units in the cytoplasm, specific protein differences probably distinguish them, but the conception is no more than hypothetical. The lipoids are known to be concentrated in mitochondria and probably in films or membranes. More complete segregation of a single recognizable chemical substance is associated with *products* of the cell, such as food material, excreta and cell walls, rather than with the labile protoplasm itself.

Along with organization of structure in the cell goes mobilization of energy, and the foregoing sketch of the structure of the cell is only preliminary to a more detailed study of its dynamics. This is attacked by considering in turn the various types of physical force that are known to act in cells, first with reference simply to physical systems analogous to living ones and then with reference to the cell itself. It is convenient to begin with forces that act at surfaces since this particularly demands further observation of cell structure. From this we pass to the study of forces which act predominantly in solutions—conjoined with such structural features as occur in cells—and finally to the study of colloidal substances in which the properties of surfaces and of solutions unite to impart special characters that are also conspicuous in the living substance.

The Importance of Surfaces in Vital Phenomena

Organisms are made up of microscopic cells, cells of various lesser parts, and these again of ultramicroscopic or invisible colloidal particles. Each subdivision involves greater surface. If we estimate that the particles have a mean diameter of 5$\mu\mu$ and that altogether they total one-fifth of the volume of our bodies, each of us contains about 30,000 square meters of internal surface. This fact is of importance because free energy resides at interfaces, energy which displays itself among other ways in those active movements and in that catalysis of chemical reactions which are among the most characteristic features of life. The operation of surface forces appears as two main types of physical phenomena—Surface Tension and Adsorption—which are now to be studied in turn.

CHAPTER III

SURFACE TENSION IN PHYSICAL SYSTEMS

I. STATIC CONDITIONS

1. Evidence of Tension in Surface Films.—That the surface film of a liquid has different properties from the interior is shown for example by the fact that a needle will float on the surface of water although it readily sinks once it is submerged. A film of air prevents the water from wetting the needle, which, if carefully placed on the surface, lies in a trough and evidently exerts a pull on the surface of the water. The well-known rule that a liquid surface assumes the least area possible under the circumstances leads also to the conception that it possesses a tension as of an elastic membrane tending to reduce area. Neglecting the thickness of the film the force is regarded as acting across a certain length. Its dimensions are $\frac{\text{force}}{\text{length}}$ usually expressed as $\frac{\text{dynes}}{\text{centimeters}}$. There is one important respect, however, in which surface tension in a liquid differs from that of an elastic membrane. The latter is proportional to stretch, the former is unaltered by increase of surface.

2. Figures of Minimal Area.—There are many shapes other than the sphere which may indicate that a body of liquid is moulded by surface tension. The least area that can enclose a given volume of space is a sphere, the form approximately assumed by a free soap bubble or small drop. But if a soap film is in contact with a solid support, the number of shapes it can be made to assume is indefinite depending on the shape of the support. Yet all are figures of minimal area and one law governs all, namely, the law of *constant mean curvature* at all points. The curvature varies as the reciprocal of the radius of curvature. The mean curvature is the mean of the curvatures along two planes at right angles to one another. For example, if a soap bubble is drawn out along

one axis it assumes a shape such as shown in Fig. 6. The horizontal radius of curvature (r_1) varies according to the vertical position and so does the vertical radius (r_2). Near the end the vertical curvature even becomes negative, but the horizontal is correspondingly increased. At all points $\frac{1}{r_1} + \frac{1}{r_2}$ is constant.

The mean curvature is constant because it depends on the difference of pressure inside and outside the bubble and this is the same at all points. If the pressure is equal on both sides of a film the mean curvature is zero.

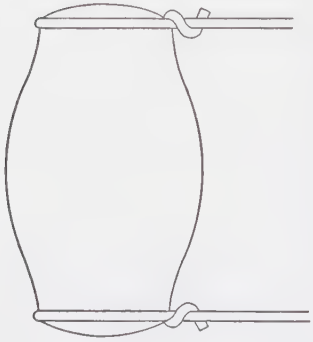

FIG. 6.

The above remarks apply to liquid drops as well as to soap bubbles except that the greater weight of the liquid causes a greater divergence from the ideal shape. With microscopic masses of liquid, however, such as we deal with in the cell, the effect of gravity is negligible.

3. Angles of Contact.—When three media instead of two are involved, three interfaces meet. These set themselves so that the forces are in equilibrium.

Two Liquids and a Gas.—When a drop of paraffin (nujol) is placed on water it assumes a lenticular form, Fig. 7. The three interfaces set themselves so as to satisfy the rule of the triangle of forces. If one adds soap to the water the drop contracts, that is, it tends more toward the spherical shape because the distorting tension of the water-air interface is reduced. If one adds mastic to the oil, the drop widens because the oil-water tension is reduced.

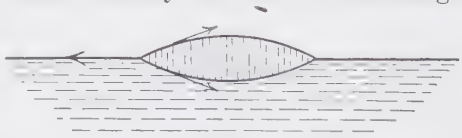

FIG. 7.

Liquid, Solid and Gas.—The angle θ which the liquid makes with the solid is called the angle of contact. Equilibrium exists when

$$T_{sa} = T_{sl} + T_{la} \operatorname{Cos} \theta$$

If

$$T_{sl} = T_{sa} + T_{la}$$

θ becomes 180°. There is no wetting and no adhesion between liquid and solid.

If

$$T_{sa} = T_{sl} + T_{la}$$

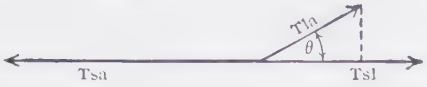

FIG. 8.

θ becomes zero with complete wetting. The degree of wetting and of adhesion varies inversely as the angle θ.

$T_{sa} - T_{sl}$ or $T_{la}\,\mathrm{Cos}\,\theta$ is sometimes called the Adhesion tension (Haftspannung).

4. Measurement of Surface Tension and Surface Energy.—As a result of the force which tends to reduce surface, there resides at surfaces a special kind of energy. The relation between surface energy and surface tension σ is simple. Maxwell's exposition makes it clear thus: Imagine a rectangular wire frame of which one side CD can slide freely along the two adjacent. The arrangement is vertical with a soap film enclosed by $ABDC$. A weight attached to CD if just balancing the tension on the film will maintain the wire CD at any position irrespective of the area of the film (within limits). Since there are two surfaces it equals double the surface tension.

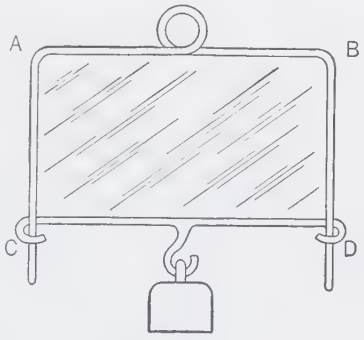

FIG 9.

The free energy in the film depends on the work it can do in contracting to the minimum, that is, in lifting the weight through the distance AC. The force is the surface tension (σ) per unit length $\times$ length CD. The free surface energy = force $\times$ distance = $\sigma \cdot CD \times AC$ = surface tension $\times$ area of surface. The free energy per unit area is thus numerically equal to σ.

There are various methods of measuring surface tension. One of the most convenient for biological purposes, though not the most unobjectionable, depends on the adhesion of the liquid to a plat-

inum ring. The principle of the method is the same as that of Maxwell's model just described. The details are explained in the practical part.

5. Cause of Surface Tension. (*a*) *Molecular Forces at a Liquid-Air Surface.*—Although surface tension is measured as a tension it is desirable for many purposes to regard it from the point of view of the molecular forces to which it is due. The cause of the phenomenon is the unbalanced force acting on the layer of molecules next a surface. A molecule in the interior of a liquid is equally attracted on all sides. Molecules at a free surface are only attracted inward, therefore they tend to move inward; the maximum number leave the surface and the surface assumes minimal area. With this mechanism in mind we can better understand surface tension relations at an interface between two liquids or between a liquid and a solid, and also the effect of dissolved substances.

(*b*) *Molecular Forces at Interfaces.*—Call the media A and B. The unbalanced force acting on a molecule in the surface of A is $A \longleftrightarrow A - B \longleftrightarrow A$. In the case of air $B \longleftrightarrow A$ is negligible. With another liquid or a solid it has a certain value, thus reducing the interfacial tension below water-air tension. Similarly the unbalanced force in the surface of B is $B \longleftrightarrow B - B \longleftrightarrow A$. The interfacial tension depends on the sum of these: $A \longleftrightarrow A + B \longleftrightarrow B - 2\,(B \longleftrightarrow A)$, or cohesion of A + cohesion of B − twice the adhesion of A to B. As this approaches zero so does the interfacial tension, and finally instead of assuming minimal area the liquids mix in all proportions. Short of this, however, there is always a certain amount of molecular mixing (solution) on account of thermal agitation (kinetic activity).

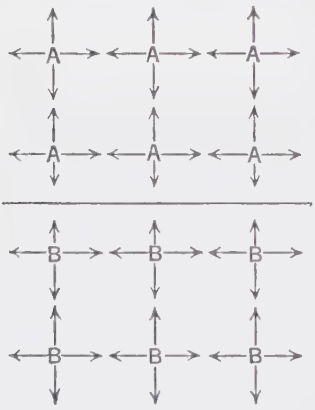

Fig. 10.

(*c*) *Molecular Forces in Solutions.*—When a substance (S) is added to water (W) its effect on the water-air tension depends on $W \longleftrightarrow S$ as compared with $W \longleftrightarrow W$. If the former is the weaker the cohesion of the liquid and still more its surface ten-

sion are lowered. If it is the stronger these are raised. But in contact with a medium B which is either liquid or solid the attraction $B \longleftrightarrow S$ may largely overcome $W \longleftrightarrow S$, and produce a different effect. Thus many inorganic salts which raise the water-air tension lower the tension at the interface of water with some other medium such as a hydrocarbon oil. On the other hand if S dissolves in B we may find that eventually it has less effect on the interfacial tension than on the surface tension. This is because its concentration in the water is reduced and therefore the surface tension of the water side of the interface—which is the principal element in the combined interfacial tension—goes up. Thus fatty acids are less active than soaps at a water-oil interface, though not at a water-air interface, because the acids dissolve in the oil.

The relation of solute to solvent must always play a part in interfacial tension and very commonly it is the major part. The order of activity of solutes at the water-air surface is often paralleled at interfaces and demands some further discussion.

6. Capillary Activity in Relation to Chemical Structure.—Although the distinction is arbitrary it is useful to classify substances according as they are capillary (or surface) inactive in aqueous solution, that is, have little effect on surface tension, or capillary active, that is, greatly lower it. The former group includes all those that raise the tension because they never do so to any great extent, whereas substances which have a very slight depressing effect or no measurable influence at all are generally more nearly related to those that raise tension than to those that markedly lower it. Since this distinction between surface activity and surface inactivity will be found to appear again and again in classifying substances according to their physiological or pharmacological action, it is well at this stage to get some idea of the more general physical and chemical characters that appear to determine it.

As we have seen, it depends mainly on the attraction between the solute molecules and water. Not enough is known of these molecular forces to deduce quantitatively any property of a solution from them, but certain generalizations are made as to the relation between chemical constitution and hydrophily. The most general rule of molecular attraction is that polar molecules strongly attract one another and non-polar molecules also

attract one another, whereas polar and non-polar have little attraction.

The polarity referred to is electrical. Some molecules are (or have the power of becoming) minute magnets; others have not this property. Also there are all degrees of polarity. To understand fully how polarity is brought about, one would have to know the grouping of atoms in the molecule and the relations of protons and electrons in the various atoms. These protons and electrons (the elementary constituents of which an atom is made up) are units of positive and negative electricity respectively. If the " center of gravity " of all the protons coincides with that of the electrons the combination is not a magnet; it is non-polar. In proportion as the centers are separated the molecule is polar. The same applies to an atom or group of atoms within the molecule. It may be that the separation takes place only in an electrical field, but the point is that proximity of other potentially polar molecules has the effect of setting up polarity in both, so that they attract one another—like magnetized iron filings—at their unlike poles. The attraction between non-polar and polar molecules and also between non-polar molecules themselves is of a lower order because it is deficient in this magnetic quality. The most strongly polar molecules tend to ionize when dissolved in a suitable medium but many practically undissociated substances, mainly those rich in *OH* groups, such as alcohols and carbohydrates, are also classed as polar. Water is typically polar. For a typically non-polar solvent we must go to organic compounds, that is, the hydrocarbons, either ring or chain. The ring compounds, such as benzol, are the better solvents.

To return to the question of capillary activity, it is evident from what has been said that the more polar a solute, the greater will be its attraction for the polar molecules of water and the greater its tendency to raise or maintain surface tension when dissolved in water. Inorganic salts, the most polar of all substances, appreciably raise surface tension. To a certain extent solubility in water also follows polarity as we might expect, but the factors governing solubility are more complicated than those which determine surface activity in a dilute solution.

Similarly it follows that the less polar a substance the less will be its attraction for water and the greater its surface activity, if it is water-soluble at all. Again there is a less complete symbasis

between insolubility in water and solubility in non-polar solvents such as benzol.

Most organic compounds consist of a non-polar portion, for example, a hydrocarbon radical, united with a polar group or groups such as COOH, NH_2, OH. Thus a fatty acid combines characters of both classes of substance. One with a long hydrocarbon chain shows predominantly non-polar characters such as solubility in organic solvents and high capillary activity in water, if at all soluble therein. One with a short chain shows predominantly polar characters, solubility in water and weak capillary activity.

Traube's Rule.—Traube discovered a quantitative relation between length of carbon chain and capillary activity. In a homologous series of carbon compounds surface activity increases strongly and regularly as we ascend the series. To obtain the same lowering we need of each successive member about one-third of the concentration of the previous member with one CH_2 less. The usefulness of this rule will appear later.

7. Structure of Surface Films.—The asymmetry of form and the polarity of forces displayed by most molecules tend to bring about their definite orientation with respect to one another whenever their kinetic energy is not too high. At surfaces and phase boundaries the orientating forces come particularly into play.

The most striking experimental proof of orientation concerns insoluble substances which can form films on water. A drop of benzol solution, for example, of a fatty acid spreads on the surface of water; the benzol evaporates and leaves the acid as a thin film which may be liquid or solid. The area of the film can be measured and its thickness is the volume of solid substance divided by the area. The thickness varies directly as the length of the carbon chain in the fatty acid molecule and is of the order of magnitude to be expected of a layer of molecules *set on end.* The area occupied by a single molecule calculated from the number of molecules (derived from Avogadro's constant) also agrees with this conception. Langmuir—who led the way in this field of research—explains the perpendicular orientation by the attraction for water of the polar group COOH at one end of the carbon chain.

It is noticeable that the surface tension of the pure liquids of a homologous series of organic compounds does not vary greatly

although their capillary activity in solution does. This is taken to mean that these also have the superficial layer orientated with CH_3 groups pointing outward.

8. "Negative" Surface Tension: "Myelin Forms."—If lecithin is allowed to swell in water it extends into the medium in the form of contorted tubes showing a laminated structure—the so-called "myelin forms." In other words, it apparently tends as rapidly as its viscosity will allow to form films of the *maximum* area. This is contrary to anything that we should expect from the simple theory of surface tension which treats of molecules as if they were entirely symmetrical spheres. On this theory either minimal area tends to be assumed, or, if surface tension is zero, complete mixing. The simple theory fails to take account of the effect of asymmetry in the fields of force around molecules as described in the preceding paragraphs. Molecules may find their position of greatest equilibrium by close packing in an orientated film and the reduction of free energy in that way may be greater than that attained in the reduction of surface. Whether we are to call this condition real or only apparent negative surface tension depends on definition. Probably the tendency of lecithin in water is toward a bi-molecular layer with the polar groups pointing outward on each side (at the surface of the water and air it forms monomolecular films). Shaking breaks up the films and leads to a colloidal dispersion of the lecithin.

Few substances in contact with water are so delicately poised between minimal area and mixing, but those that exhibit this condition—the phospholipins—are common if not universal constituents of protoplasm.

II. KINETIC CONDITIONS

1. Causes of Change in Surface Tension.—In the foregoing sections we have considered the cause of surface tension and also some of its results when conditions are static, that is, when there is an equilibrium of forces. If the equilibirum is upset, either by a change in surface tension or in factors which oppose its action, movement commonly ensues. Of the factors which *alter* surface tension the commonest is a *chemical change*, principally the addition or withdrawal of capillary active substances, the nature of which has already been described. *Change of temperature* also

modifies surface tension, varying inversely, but the variation is too slight to be of importance within the range of temperatures at which life can exist. There may, however, be greater indirect effects, especially through change in viscosity.

Of the factors which *oppose* the action of surface tension, *electric charge* and *vicosity* or *rigidity* are the chief. The repulsive action of like charges tends to expand a surface and thus to reduce its apparent surface tension. Since surface charge is readily modified by electrolytes it is liable to frequent variation at protoplasmic surfaces. Changes in physical state, however, probably play the greatest part of all in determining to what extent and with what rapidity protoplasm assumes minimal area. The more mobile the liquid, the easier is it for surface tension to mould its shape. Viscid liquids on the contrary can be drawn into threads or beaten into a froth because the internal friction in a thin layer is too great for their surface tension to overcome.

2. Movements Resulting from Change of Surface Tension.—(*a*) Consider the first condition when the tension over a whole surface is changed uniformly. For example, when three liquid surfaces are set so as to be in equilibrium and the surface tension of one is changed, the readjustment of angles of contact that is required to bring them more into equilibrium involves temporary movement and change of shape. Thus if a lenticular drop of oil lies on a water surface and the water is touched by soap the oil drop widens, but if gum arabic is added to the oil it contracts.

(*b*) Another case is that of local change in surface tension, that is, over part of a surface. For several reasons uniformity of surface tension tends to be reestablished unless the disturbing agent continues to act, but in the latter event continuous movement may result. Thus a drop of water on glass may continue to retreat before alcohol, ether, or the vapours of these. On the side next the applied agent the angle of contact of the water is higher than where the third medium is air, so that the water tends to spread on the air side and withdraw on the alcohol side.

Apart from changes in the angle of contact certain other movements result from local changes in a single surface. Consider what will happen in the simplest case—that of a spherical drop of one liquid suspended in another—if the surface tension is lowered over a certain area. To begin with there always exists a certain *excess of pressure inside* over that outside any drop owing

to the tension of its surface. This hydrostatic pressure being *equally transmitted* throughout the body of the drop, if the tension, or more fundamentally the centripetal molecular attraction of the surface, is anywhere *reduced relative to the rest* (indicated in Fig. 11 by a negative sign), that region will be forced to *bulge out* like the weak spot in a toy balloon. Unlike an elastic balloon, however, the opposing tension will not be increased by stretching. What then will set a limit to the local bulging of the drop? The smaller the radius of curvature the greater the vertical component of the surface tension force. Now the bulging of any region less than the hemisphere will tend to produce a new portion of a sphere of smaller radius than the parent up to the production of a new hemispherical protuberance. (Of course the junction of the two spherical areas will adjust itself to a graded curvature.) It is this increase of curvature or decrease of radius of curvature which will finally allow the centripetal force in the region of lowered specific surface tension to balance the internal pressure. This behaviour, as well as other effects of a local lowering of surface tension, is exemplified in the experiment with a drop of Hg and a crystal of $K_2Cr_2O_7$ in nitric acid. The action of the dichromate is apparently to lower the surface tension of the Hg drop in its vicinity. This produces manifold results: (1) a pseudopodial outflow of the Hg toward the crystal as explained above; (2) a bodily movement of the Hg drop in the same direction caused by the flow of liquid from the region where it is heaped up (larger radius of curvature) to where its upper level has fallen with the pseudopodial outthrust (smaller radius); (3) the production of currents near the interface both in the Hg and in the surrounding medium as evidenced by the movements of the red oxide of Hg which is produced in the reaction.

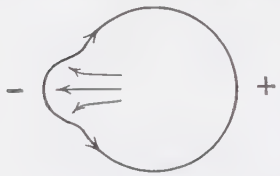

Fig. 11.

The origin and outcome of these currents require a moment's consideration. They are due to tangential movement of the surface film. We have seen that when soap is applied to a water surface any small object floating on the water is caused to move away from the point of application for the reason that surface tension is lowered on the nearer side before the soapy surface film has time to envelop the object completely. Similarly in the " Red Sea "

trick, in which a brush wet with alcohol is applied to a thin layer of water, if visible particles as of carmine are suspended in the water, it may be seen that circulating currents are set up by contact with the alcohol. The surface film, as always, moves away from the region where solution of the alcohol is lowering tension and this drags along by friction some underlying layers of liquid; a back current in the interior brings fresh water to the alcohol-water interface and helps to complete the cycle. The currents which were noted in the vicinity of the acid-mercury interface have a similar origin.

One general result of this tangential movement of the surface film is the tendency to rapid restoration of uniformity of surface tension. Whatever the local change in the surface film by which the alteration of surface tension is produced, this change will tend to be transported along with the movement of the film itself to other parts of the surface. Even if there is continued production of the cause of change in one locality, saturation of the film is often speedily attained, as when the application of soap produces only a brief movement of a floating needle although solution of the soap in the body of the liquid still goes on. In order that a continuous movement may go on with local lowering of surface tension as the prime physical cause, not only must there be continued production of the chemical or other cause at the locus in question, but continued annulment of it in other regions of the surface to which it is so readily transported.

CHAPTER IV

SURFACE TENSION IN CELLS

I. STATIC CONDITIONS. –The Shapes of Cells and Cell Organs

The main theme of this chapter is the part played by surface tension in determining cell morphology. This reacts however on experimental cell physiology because one of the readiest clues to changes in the physical state or respectively the surface tension of protoplasm is change in its tendency toward minimal area. It gives us an alternative. A reduction of surface may mean either a fall of vicosity or an increase of surface tension or both. One must search for some collateral evidence as to which has taken place.

1. Figures of Minimal Area in Cells and Cell Parts.—The most elementary knowledge of what constitutes a figure of minimal area is sufficient to teach us that the spherical form commonly assumed, for example, by a resting *Amoeba*, a plasmolyzed protoplast, and the protoplasmic masses extruded from the cut end of e. g. a *Vaucheria* filament, or, within the cell, by small vacuoles, by the nucleolus, and frequently by the nucleus, are all determined by surface tension. A little further knowledge of the contours that obey the principle of constant mean curvature and therefore of minimal area, tells us that many of the beautiful and symmetrical figures other than spheres assumed by free living cells are such as might be moulded by the same force modified by some other factor. Such figures are the hanging drop or flask shapes common among *Infusoria* (Fig. 12*a*, *Salpingoeca; d*, *Folliculina*), and the funnel shapes (such as might be assumed by a soap film connecting two unequal rings) of *Vorticella* (*c*). Sometimes a cell,

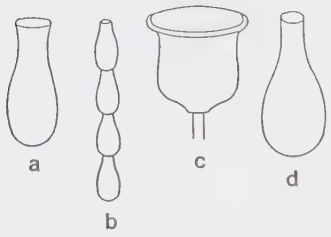

FIG. 12.

naked at first, becomes invested later by a rigid covering but retains the shape imposed upon it while the free surface was still fluid, for example, calcareous foraminfera and the spores of many plants. Among the foraminfers, as in *Nodosaria* (*b*), we may also find a series of hanging drops each exuded from the one above after the latter's shell has formed.

Plasmolyzed protoplasts of elongated cells when they contract away from the wall assume similar " unduloid " forms usually appearing as a chain of beads large and small, connected by threads. This form is partly the result of the initial shape imposed upon the protoplast by its cell wall and partly the effect of viscosity. A heavy oil may be pulled out under water into similar forms. The lenticular outline assumed by the nucleus in most plant cells is the combined result of surface tension and lateral pressure. In *Spirogyra* the secondary factor is the tension of the suspending threads of cytoplasm. If these threads break down or if the nucleus escapes into the sap vacuole it immediately becomes a sphere.

It is therefore possible to recognize in a great many of the varied forms assumed by free surfaces of protoplasm merely the action of a physical force, surface tension, in combination with some other simple factor such as contact with a solid, the force of gravity, or the viscosity of the protoplasm itself.

It is much more difficult to identify minimal area figures in the shapes of cells in aggregate. We are helped by a study of soap bubbles in groups and in foams. A layer of equal bubbles resting on a flat surface makes in the plane of contact a regular pattern of hexagons with tiny intercellular spaces where three films meet, altogether resembling the cross-section of parenchyma tissue in a plant. When the bubbles are of different sizes the smaller ones bulge into the larger and the angles between adjacent films departs from 120°, as is also illustrated in tissues.

The theoretical three-dimensional shape of equal bubbles in a foam has been a puzzle to the mathematicians. In experiment it varies because bubbles like cells are never exactly equal in size, but the general resemblance at least of say a segmenting egg to a foam is very striking. In plant cells the rigid walls, the pressures and the unequal growth often induce shapes far divergent from minimal area. Yet, when first formed, every wall is liquid or plastic so that **Errera's rule** holds good on the whole. The rule is

that " at the time of its inception every cellular membrane takes the form and position which would be assumed under the same circumstances by a weightless liquid film." We must at the same time recognize in dealing with cells possessing an elastic membrane that such envelopes under the turgor pressure of the cell also tend, though less perfectly than fluids, toward minimal area. For a wealth of examples of surface tension figures in cells the student is referred to Thompson's " Growth and Form."

2. Non-Minimal Area in Fluid Protoplasm.—But after all, the surprising thing is not that fluid protoplasm often shows figures of minimal area but that it so often does not. It is more common to find naked protozoa irregular, often highly so, and with pseudopodia rather than rounded and smooth, for example, *Allogromia* (Fig. 13). The vacuole of a plant cell is commonly traversed by strands of protoplasm—frequently better described as "streams" since they flow from side to side; the general vacuolar surface instead of being smooth is sometimes a medusoid growth of writhing filaments; the kinoplasm, streaming or not, runs like a maze through the protoplasmic matrix; and the chloroplasts of many algae, such as *Spirogyra*, have a highly anfractuous outline. Minimal area appears to be the exception rather than the rule in the protoplasmic interfaces of an active cell. There are three explanatory causes of non-minimal area: viscosity, negative surface tension and unequal surface tension.

Fig. 13.—(From Calkins.)

Viscosity.—The first is invariably at play. Protoplasm is not a mobile liquid. Its viscosity is often high; it is commonly elastic as well as plastic, a jelly as much as liquid. At any rate the viscosity or rigidity is often too great for the normally low interfacial tensions to overcome. Experiments show, however, that the balance is a delicate one. An electric shock or a trace of $BaCl_2$ upsets it completely in a *Spirogyra* cell. Either surface tension is raised (by reduction of charge) or viscosity is reduced—probably both—and the vastly extended surfaces shrink to a

minimum, so that without loss of life the topography of the cell is unrecognizably transformed. Life, it has been said, resists physical force. It would be more correct to say that life maintains a balance of complex forces.

Negative Surface Tension.—The second explanation is hypothetical. It is that protoplasmic surfaces may possess the same property as produces the myelin forms of lecithin—sometimes described as "negative" surface tension. The hypothesis is rendered plausible on account of the probable presence of lecithin or other phospholipins in protoplasmic films.

Fig. 14.—Diagram of a normal cell of Spirogyra porticalis and of stages in reduction of internal surfaces toward minimal area induced by passage of electric current.

Unequal Surface Tension.—The third explanation of departure from least surface is that life is dynamic. Chemical changes go on continuously and also with local limitation in a living cell. Hence arise local differences of surface tension on the same morphological surface. This however always results in a changing, never a static, condition. Irregularities due to inequalities of surface tension continually alter their shape and will be dealt with more fully in the next section.

II. KINETIC CONDITIONS.—Protoplasmic Movement

Protoplasmic movement is of great variety. Many forms of it have a deeper-seated origin than change of surface tension at visible surfaces. The subject will again be taken up in dealing with colloids. At this stage, however, we shall examine those changes of shape that bear a resemblance to the surface tension models which we have already studied.

1. Amoeboid Movement.—The question to be decided is whether the mechanism of amoeboid movement is really the same as in the physical behaviours which simulate it or whether the resemblance is purely apparent. It is only by closely analyzing the happenings in both cases that we shall be able to make a useful comparision. That the so-called amoeboid movements of the

lifeless models are caused by local changes in surface tension follows both from the agents which involve them and from the behaviours themselves. The latter, as both theory and the experiments performed indicate, are somewhat as follows:

Given a liquid drop resting on a solid surface and suppose the surface tension of the drop to be lowered on the side *A*.

(1) *Only the free surface of the drop affected;* The following series of changes ensues:

The drop protrudes over its area of low tension. (Example—an olive drop touched by KOH or Hg in HNO_3 by a crystal of $K_2Cr_2O_7$.)

The angle of contact of *A* alters. If less than 90° it will be reduced and the drop will tend to spread on that side (Fig. 15, *a*). If greater than 90° it will increase, but in spite of this the "pseudopodium" may protrude on the side *A* (Fig. 15, *b*). (Example—Changes in the angle of contact of water and glass in presence and absence of vapours of alcohol, ether, etc.)

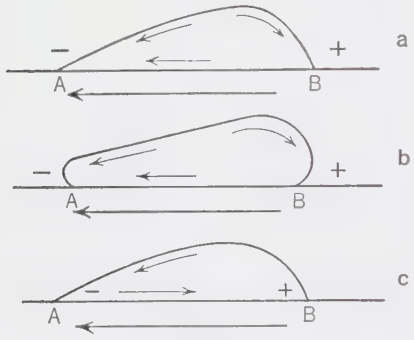

Fig. 15.

To maintain the angle of contact at *B* and to compensate for the flow of liquid towards *A*, the margin *B* retracts. Thus bodily locomotion of the drop ensues (as in the mercury drop).

The surface film moves from the region of low to that of high tension setting up circulatory currents in the drop. This tends to restore a uniform surface tension over the surface of the drop unless continual lowering goes on at side *A* and raising at side *B*.

(2) *Only the surface of contact of drop with substratum near A affected;* Result—A lower angle of contact and spreading on side *A*. Compensating retraction on side *B* (Fig. 15, *c*).

(3) *Both of the above surfaces affected on side A;* Result—A combination of the above results, and movement toward *A* is enhanced. In all cases of course a new position of equilibrium tends to be established unless continual work is performed. Otherwise we should have a perpetual motion machine.

Turning to amoeboid cells we find that there are many different types of movement. Sometimes an out-thrusting pseudopodium exhibits a low angle of contact, sometimes a high. Some kinds of *Amoeba* during locomotion thin out toward their anterior end to a hyaline film and are piled up posteriorly. Their profile is essentially that of the drops in Fig. 15, *a* and *c*. As will be shown

later, they also ingest their food by sliding over it or sucking it in with a low contact angle. It is plain that these movements are, partly at least, produced by the same mechanism as in the physical models.

More commonly perhaps these wetting movements are fluctuating and non-directional, bearing no relation to locomotion. Waves of extended hyaloplasm (the clear external layer of an amoeba) may sweep aimlessly around the margin of a stationary cell or one which is advancing steadily in one direction by a totally different mechanism. These movements may be exhibited even by dead or cytolizing cells. Moreover, certain amoebocytes in the blood of invertebrates spread to such an extent on a glass surface that they become flattened out in a thin film to their own destruction. In all such cases the superficial changes in tension are evidently accidental. The mechanism of locomotion lies deeper.

In typical amoebid movement, and possibly in all forms of it, locomotion is accompanied by vortical streaming of the granular protoplasm (Fig. 16). In the axis of the pseudopod or of the whole organism the stream flows forward. Toward the periphery it flows backward if the cell is suspended in liquid, or remains stationary if it adheres to a substratum. In the latter case the whole mass moves forward by continually turning itself inside out. So far the behaviour resembles that of a drop in which the surface tension is lowered at the advancing end. But the behaviour of the superficial hyaline layer is contrary to this assumption. Particles adhering to the surface move slowly toward, not away from, the anterior end, that is, in the direction opposite to that of the granular ectoplasm immediately beneath.

Fig. 16.

Two explanations that have been suggested of this peculiar phenomenon may be mentioned. One assumes an interface between the hyaloplasm and the granular plasma at which changes of surface tension take place causing the amoeba lying in its pellicle to move by the same mechanism as the simple physical model. The other explanation assumes that the surface tension of the hyaloplasm is actually higher anteriorly and ascribes the pseudopod formation and movement to colloidal changes throughout the cell. This view is supported by the fact that notable

variations in viscosity mark the several stages of the cycle of streaming. Fundamentally these colloidal changes may also be due to surface tension; but the latter's mode of action in a collodial system must be reserved for a later chapter.

2. Plasma Streaming.—This appears to fall into the same category as amoeboid movement. Streams of kinoplasm within the cytoplasm resemble the fine pseudopodia and streaming filaments which arise from the vacuolar surface of plant cells or the external surface of naked cells. The more massive type of streaming in *Chara* and *Nitella* has been the subject of a good deal of experimentation. Here a thick inner layer moves past a thin stationary layer of protoplasm adjoining the wall. The latest results indicate that the driving force resides at the boundary of these two layers, but whether there is a definite interface at which surface tension might act is uncertain.

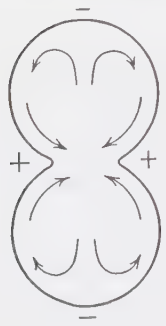

Fig. 17.

3. Cell Cleavage.—When a drop of olive oil, mixed with chloroform till it sinks, is immersed in water and touched at opposite poles by crystals of soda, the drop constricts at the equator and may divide into two. The tension, being lowered at the poles, is relatively higher in the region of constriction; in consequence the surface film moves toward the furrow and vortex currents are set up. All these behaviours are duplicated in cell cleavage, as in dividing eggs, pointing to a similar surface tension mechanism.

4. Ingestion and Excretion.—A practical experiment shows that if a small glass rod is pressed lightly against a chloroform drop lying in water the drop is dimpled in, refusing to wet the glass. Also if the rod is pushed partially into the drop and then released, it is frequently thrown out. On the other hand, if the glass is coated with shellac, the chloroform spreads over it or draws it in as the case may be.

The three stages which we have distinguished in the spreading of a liquid over a fixed solid are duplicated in the ingestion or egestion by a liquid of a movable solid particle, and are as in Fig. 18:

1. Non-adhesion = non-ingestion: *A*.
2. Adhesion with partial wetting = partial ingestion: *B*, *C*, *D*.
3. Adhesion with complete wetting = complete ingestion: *E*.

The respective angles of contact are 180 deg., between 180 and 0 deg., and 0 deg. In many cases of ingestion by *Amoeba* the angle of contact seems to be zero. A filamentous alga may be pulled in and coiled up within an amoeba (Fig. 19) by apparently the same mechanism as with a thread of shellac and a drop of chloroform.

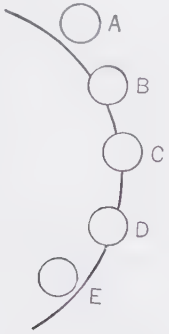

Fig. 18.

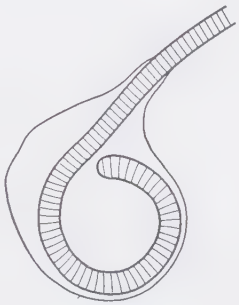

Fig. 19.

Similarly leucocytes have been observed to ingest bacteria by wetting. Complete ingestion, however, is not necessary for digestion. Some amoebae digest their prey when only in contact with one side of it, and it may be that leucocytes can similarly attack bacteria. But at least some degree of adhesion is probably essential in phagocytosis. The bacteria are safe unless they stick to the phagocyte. The ability of higher animals to resist the attack of pathogenic bacteria depends mainly on the ability of the leucocytes to ingest these. This in turn depends on surface tension relations. As has just been indicated low tension (or high adhesion) at the interface between leucocyte and bacterium leads to the destruction of the parasites and establishes immunity. Substances termed **opsonins** which increase phagocytosis are produced in the blood in the course of the develop-

ment of an immunity. They probably alter surface tension in the way indicated.

Among the *Rhizopoda* (amoeba, etc.) the ingestive, like the locomotory, mechanism is very varied and is probably not always a case of low contact angle. Amoebae are said to flow around a food particle without actually touching it, a quantum of water being included in the meal. Here it would seem that some chemotactic influence coming from the particle locally raised the surface tension of the amoeba.

Fig. 20.

The excretion of solid particles is the reverse of their ingestion and need not be specially discussed. In our physical model a glass rod is ejected when its coat of shellac is dissolved by the chloroform. The excretion of water, of solutes and also to some extent of suspended particles seems to be largely effected in another way, namely, by the formation and bursting of vacuoles. This behaviour has long been recognized among the *Protozoa* especially those living in fresh water, the low osmotic pressure of which causes them to absorb water rapidly by omosis and thus imposes the necessity of getting rid of it in some other way. This they do by means of so-called " contractile " vacuoles. In cells where there is a rigid cell wall to oppose the internal pressure, excretion of water is unnecessary, but *Spirogyra* gametes during conjugation are under the same necessity as a naked cell and meet it in the same way. It is probable that solutes as well as water are thrown out. At least the excretory vacuoles have a certain osmotic pressure while that of the central vacuole goes down steadily during the excretion.

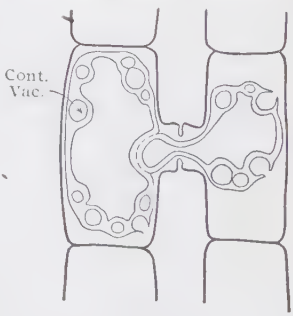

Fig. 21.

We know little as to the physical mechanism of the formation and growth of contractile vacuoles but infer from their behaviour that their evacuation is probably governed by the same simple laws of surface tension and viscosity as determine the bursting of a soap bubble. The higher the surface tension and the lower the viscosity of the film separating the vacuole from the exterior, the more readily will it

burst. In *Spirogyra* we must infer that these conditions obtain more fully at the external than at the vacuolar surface; otherwise the contractile vacuole would discharge itself into the central vacuole.

In gland cells of higher animals, at least in kidney, thyroid, and epididymis, secretory vacuoles have also been recognized as playing an important part. It is a point of practical interest, though experimentally undeveloped, that the normal functioning of these organs must therefore depend markedly upon the physical state and surface tension of their cells. The surface tension relation of cells at an oil-water interface has been used[1] to give information about the character of their surface. Closely allied types may differ markedly. Thus some kinds of bacteria pass into the oil, others do not. (The two classes differ also in staining property.) If the surface of bacteria that pass into oil (tubercle bacilli) is extracted with alcohol or coated with substance in the proper immune serum (see p. 58) they no longer have an affinity for the oil.

[1] Mudd, S., and Mudd, E. B. H. (see Alexander, p. 437).

CHAPTER V

ADSORPTION IN PHYSICAL SYSTEMS

Adsorption is the name given to the concentration of substance at a surface under the action of forces residing there. Frequently a distinction is made, according to the nature of these forces, into *mechanical*, *electrical*, and *chemical* adsorption. **Mechanical** adsorption is produced by those molecular forces that appear also in surface tension. **Electrical** adsorption by the attraction of unlike charges on ions. **Chemical** adsorption by the forces of ordinary chemical combination.

The last, involving as it does a change in the chemical nature of the adsorbed substance rather than in its mere concentration, is often excluded. In many cases, however, it is impossible to decide whether it is mechanical or electrical, which is not surprising since at bottom all are probably electrical by nature.

Much of the theory of the subject is based on a study of the adsorption of gases but for practical purposes we are concerned in physiology only with adsorption from solutions and shall confine our study to this.

I. MECHANICAL ADSORPTION

1. Composition of Surface Films.—In pure liquids the effects of surface tension force are: (1) reduction of surface, and (2) orientation of molecules in the surface film. In solutions it may also produce a difference of *composition* between the surface layer and the interior. In the laboratory experiments the *fact* of surface concentration is demonstrated. We here consider its *cause*.

Taking first the case of a liquid-gas (say water-air) surface, if the molecules of a substance dissolved in the water are attracted to the water molecules with less force than the water molecules are to one another, the solute will tend as it were to be squeezed to the surface and will concentrate there until the opposing tendency to diffuse from high to low concentration sets a limit to the process.

Should the surface concentration reach the saturation point of solubility before this osmotic limit is reached, a crystalline or gelatinous precipitate—the so-called *haptogen membrane*[1] will be produced. When as in the above case the solute molecules in the surface layer are under a lower inward pull than the water molecules it is evident that their presence there must lower the surface tension, which as we have seen is due to this inward *pull*. Thus we see how it happens that *solutes which lower the surface tension of a solution tend to concentrate* at its surface (Gibbs' Law). Similarly solutes which raise surface tension tend to be less concentrated at the surface. But as there are no substances which greatly raise the surface tension of water, whereas many greatly lower it, surface concentration is of much greater practical importance than surface dilution.

In the case of a liquid-gas interface we may neglect the force exerted by the gaseous molecules but if the second phase is another liquid or a solid it may play an important or even the predominant part. Thus inorganic salts, although they raise the tension at a water-air surface, yet concentrate at, for example, a carbon surface. Evidently they are not squeezed to the surface by the water molecules but are pulled there by the carbon. It is to this type of surface concentration that the term *adsorption* most appropriately applies, but, as above defined, it includes that at a liquid-gas interface as well. We may also distinguish *surface concentration* as **positive adsorption** and *surface dilution* as **negative adsorption.**

2. Gibbs' Formula.—Not enough is known regarding the magnitudes of molecular forces to deduce quantitatively therefrom the relation between surface tension (σ) and concentration of solute (c). Both theory and experiment show however that if the substance is one which strongly lowers σ, or is " capillary active " as it is called, the σ–c curve drops rapidly at low concentrations and progressively more slowly as more solute is added, whereas with substances which have little effect in either raising or lowering surface tension (*capillary inactive* substances), the σ–c curve approaches a straight line. The types of curve for various grades of capillary activity are shown in Fig. 22. Once the relation between σ and c is known it is then possible on purely thermo-

[1] A membrane formed by contact—Acherson (1838); see Hatschek, "Foundations of Colloid Chemistry," 1925.

dyamical grounds to calculate the amount adsorbed at any concentration. An equation relating these quantities was first deduced by Willard Gibbs in 1906 (and is called the Gibbs adsorption formula). In general terms he pointed out how it follows from the second law of energetics that if a substance lowers the total free energy at a surface it will tend to concentrate there and if it raises the free energy will tend to leave the surface. In the simplest case, that of a dilute solution in which the adsorption is purely mechanical, that is, not electrical, the Gibbs formula which applies is:

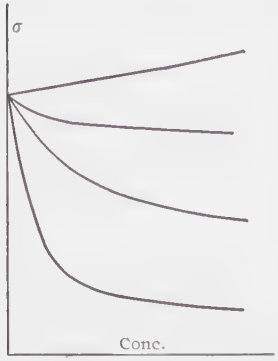

Fig. 22.

$$\mathbf{a} = \frac{c}{RT} \cdot \frac{d\sigma}{dc}$$

where **a** is the amount adsorbed or excess concentration (+ or −) in mols *per unit area* of surface;

c is the molar concentration in solution;

R is the gas constant (see under Osmotic Pressure);

T is the absolute temperature;

$\frac{d\sigma}{dc}$ is the slope of the σ–c curve.

Having plotted the σ–c curve therefore from experiment, we can also plot the **a**–c curve, since **a** varies as $c \times$ slope of the σ–c curve at that particular concentration. In practice, however, this fundamental equation is not commonly applicable in physiology since σ can rarely be measured at protoplasmic surfaces.

3. Freundlich's Formula.—A more useful formula is the purely empirical one given by Freundlich for the relation between adsorption and concentration in solution:

$$a = kc^{\frac{1}{n}}$$

where c = the concentration at equilibrium

and k and $\frac{1}{n}$ are constants for any given substance.

In practice $a = \frac{x}{m}$ where $x =$ total amount adsorbed and m = weight of absorbent, since the surface is not known. With the same uniform adsorbent a is *proportional* to the actual surface concentration (a of Gibbs' formula). Since the "constants" k and n vary somewhat with temperature the formula is termed the **adsorption isotherm.** The curve is a parabola (Fig. 23) but the adsorption relation is most readily recognized by equating the logarithms:

$$\log a = \log k c^{\frac{1}{n}}$$

$$= \log k + \frac{1}{n} \log c$$

Therefore

$$\log a \propto \log c,$$

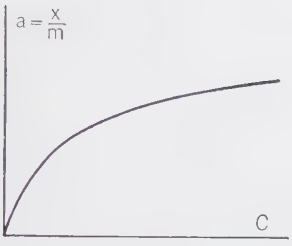

Fig. 23.

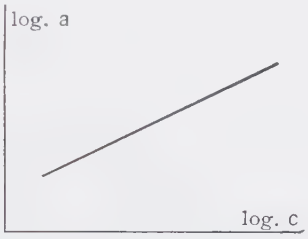

Fig. 24.

or the $\log a - \log c$ curve is a straight line (Fig. 24). The exponent $\frac{1}{n}$ is the slope of the log curve; it usually lies between 0.1 and 0.5. Freundlich's adsorption isotherm holds fairly well for dilute solutions but fails when the adsorbing surface approaches saturation point.

4. Partition v. Adsorption.—When a substance distributes itself between two phases—1 and 2—in one of which it is in solution, we may usually distinguish whether it dissolves in or is adsorbed by phase 2 by noting how the distribution varies with the concentration. If the substance exists in the same state in both phases $\frac{C_1}{C_2}$ = a constant. The $C_1 - C_2$ curve is thus a straight line, whereas, as we have seen, adsorption gives a parabolic curve. If, however, as occasionally happens, the molecules are associated or dissociated into larger or smaller units in

phase 2, then $C_1 \propto C_2^n$ where n is the relative molecular weight in phase 2.

Thus a relation $C_1 \propto C_2^{\frac{1}{2}}$ may signify either adsorption or partition with the molecules subdivided into two in phase 2. Often we shall be able to decide whether the latter alternative is possible or not.

5. Adsorption from Mixtures.—Ordinarily when two or more solutes compete for an adsorbing surface neither is adsorbed to the extent that it would be if present alone. The amount of each that is taken up depends as usual on its adsorbability and concentration, and if one is much more adsorbable than the other it may very largely displace it—preferential adsorption.

From a 0.05 M solution of glucose alone more than 50 per cent was found to be adsorbed by charcoal while under the same conditions but in presence of 0.07 M isobutyl urethane (which is strongly adsorbed) only 2 per cent of the glucose was taken up. In certain cases however the more strongly adsorbed ingredient of a mixture *entirely* displaces the others and may be as much or even more strongly taken up than in pure solution. An example is the adsorption by charcoal from a mixture of amino-acids or polypeptides of different adsorbability. In these cases adsorption depends on affinity for adsorbent rather than affinity for water. Such examples of tendency towards specificity are important as providing a step toward the still more definite specificity of adsorption exhibited by certain biological products (see later).

6. Relation to Nature of Solvent, Adsorbent and Adsorbate.—Certain surface tension relations are self evident. The higher the surface tension of the pure solvent the greater the tendency for solutes to lower that tension, the greater therefore is the tendency to adsorption from its solutions. For this reason most solutes are adsorbed in aqueous solution, fewer—and these less powerfully—in alcoholic solution. (*Cf.* washing out of stains by alcohol.) Similarly the higher the interfacial tension of the adsorbent with regard to the solvent, the greater its adsorbing power in general. Although on the other hand, between adsorbent and adsorbate a low surface tension (that is, a high attraction) favours adsorption. (That the *capacity* of the adsorbent also varies with the amount of surface it offers need hardly be mentioned.)

A more specific relation between adsorbent and solute—namely, that of opposite charge—exists as we shall see in electrical adsorp-

tion, whereas in cases where adsorption proper passes over perhaps into chemical interaction, the relation may be still more specific (for example, adsorption of iodine with a blue colouration by starch and certain other substances). As already stated, it is sometimes hard to draw the line between adsorption and chemical union. Generally, however, adsorption compounds are recognized as looser, readily reversible, more general (that is, allowing more kinds of molecules to replace one another), not stoichiometric (not showing combination in definite molecular proportions), and (probably) requiring that a large number of molecules of adsorbent simultaneously attract the substance adsorbed. They also obey the adsorption isotherm.

The difficulty of distinction between the types of adsorption can arise only when the adsorbent plays an active part. When, on the other hand, as frequently happens, there is a parallelism between adsorption at a water-air surface and at any other interface with water, it is evident that there is little specific attraction by the adsorbent. Adsorbability then becomes largely identified with capillary activity in general. There are certain regular relations between chemical character and capillary activity or adsorbability in aqueous solution which are worth noting. A high degree of adsorbability is shown by certain classes of organic substances, such as fatty acids, urethanes, aromatic acids, phenols, camphor, alkaloids, dyes, and many others. This depends partly on small attraction between molecules of the substance itself (shown in low surface tension, volatility, low melting point, etc.) and partly in small attraction for water (shown in low solubility). Among organic substance high adsorbability is associated with length of carbon chain and absence of groups such as OH which have affinity for water; in other words with **apolarity** as discussed under surface tension. **Traube's rule** again applies. With each successive member of an ascending series concentration of one-half to one-third that of the preceding one produces the same amount of adsorbate. Figure 25 shows

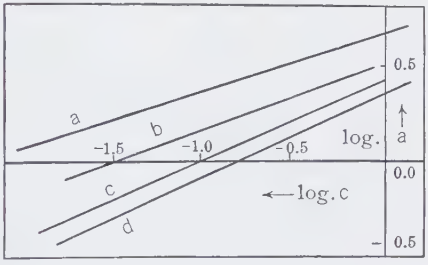

Fig. 25.—(After Freundlich.)

the adsorption curves of certain fatty acids (on charcoal) plotted logarithmically. The acids are: (*a*) butyric, (*b*) propionic, (*c*) acetic, and (*d*) formic. Other series which have been investigated and show this relation are the alcohols and urethanes.

The usefulness of the rule to the biologist is that it enables him to judge whether the pharmacological action of such series can be ascribed to adsorption.

7. Adsorption of Ions. (*a*) *Liquid-Gas Surface.*—This may be either mechanical or electrical. At a water-air interface inorganic salts raise surface tension and are negatively adsorbed; therefore in comparing the adsorbability of the various ions we must do so in terms of repulsion from the surface rather than attraction to it. Nevertheless the underlying cause of this " repulsion " is probably the attraction of water molecules for the ion. The more hydrated the ion the more does it tend to be held away from the surface. From the fact that the presence of an electrolyte usually has an effect on the electric potential of water relative to air, it follows that cations and anions are unequally " repelled." All monovalent inorganic salts make the body of the liquid more positive, indicating that the cations are repelled from the surface more strongly than the anions. The general distribution of ions near the surface may be represented as:

$$\begin{array}{ccc} Cl^- & Cl^- & Cl^- \\ K^+ & K^+ & K^+ \end{array}$$

The water molecules and H- and OH-ions are not included in the diagram. Some believe that there is a simple orientated molecular layer of water alone next to the surface.

Among the alkali cations themselves the difference is not very great but in general the order of " repulsion " from the surface follows the degree of hydration:

$$Li > Na > K > Rb > Cs \text{ followed by } Mg > Ca > Ba$$

Among the monovalent anions the difference is greater. The usual order is:

$$Cl > Br > NO_3 > I > SCN$$

Cl is preceded by anions of higher valency SO_4, HPO_4, etc.; therefore divalent salts have little effect on potential. These two series, known as the **Hofmeister** or **Lyotropic series,** crop up as we shall see in many effects of electrolytes.

(*b*) *Liquid-Liquid and Liquid-Solid Interfaces.*—At the interface between water and another immiscible liquid, salts usually lower the tension and at a solid surface they almost always do so. Evidently the positive attraction of the adsorbent is greater than that of the water. Yet the effect of the water is seen in the fact that for most kinds of adsorbents the lyotropic series still holds in large degree, especially for anions. Thus we may say that the SCN, I end of the series is more adsorbable than the Cl, SO_4 end, independent of the nature of the non-aqueous phase. Similarly the order of adsorbability of the cations tends to be:

$$Ba > Ca > Mg > Cs > Rb > K > Na > Li$$

Many other factors besides lyotropism (hydration), however, regulate the adsorbability of cations and at a charged surface that of anions also. Briefly the factors which determine the adsorbability of ions at a neutral or indifferent surface such as carbon, are:

(1) Capillary activity: Ions like molecules tend to be adsorbed in proportion to their capacity to lower surface tension and the same order is often followed at interfaces as at a water surface. This is exemplified not only with respect to the lyotropic series just mentioned but also in the strong adsorption of organic ions—an adsorption which tends to increase with the size of the ions.
(2) Valency: the higher the valency the more adsorbable.
(3) Mobility: greater mobility, greater adsorbability seen particularly in H and OH.
(4) For metal ions—position of the metal in the electromotive potential series. The nobler the metal, that is, the more strongly it holds to its electrons, the greater the adsorbability of the ions.

The following lists of cations and anions, respectively, which are in decreasing order of adsorbability by charcoal, will illustrate the composite action of the above factors at an indifferent surface.

CATIONS:— alkaloids and basic dyes $H^{\cdot}$ $Ag^{\cdot}$ $Hg^{\cdot\cdot}$ $Cu^{\cdot\cdot}$ $La^{\cdot\cdot\cdot}$ $Al^{\cdot\cdot\cdot}$ $Zn^{\cdot\cdot}$ $Mg^{\cdot\cdot}$ $Ca^{\cdot\cdot}$ $NH_4^{\cdot}$ $K^{\cdot}$ $Na^{\cdot}$

ANIONS:— various aromatic acids and acidic dyes OH' SCN' I' NO_3' Br' Cl' HPO_4'' SO_4''

Charcoal, or at least a certain kind of it, adsorbs basic and acidic dyes and also H' and OH' in equivalent amounts and thust he two series may be superimposed.

II. ELECTRICAL OR POLAR ADSORPTION

When both the adsorbent and substance in solution bear an electric charge the mutual attraction or repulsion as the case may be usually predominates over other factors in determining adsorbability. The (1) **sign of the charge** on an ion is by far the principal property determining its electrical adsorbability; usually only oppositely charged ions are adsorbed by a charged surface. The factors mentioned in mechanical adsorption of ions come into play secondarily: (2) **valency,** (3) **electromotive potential** (both of which are also electrical properties), (4) **capillary activity** and **mobility** (which are not).

Staining experiments well illustrate the importance of electric charge in adsorption. Most dyes are electrolytes, sometimes free acids or bases, more often salts with an inorganic base (Na) or acid (HCl), united to a coloured organic radicle. Those in which the coloured portion is the base are termed **basic** and those in which it is the acid are termed **acidic.** In solution the former dissociate into a large coloured cation and a Cl anion; the latter into an Na cation and a coloured anion. Methylene blue (chloride) is an example of the former; it is a basic dye. Eosin (Na eosinate) is an example of an acidic dye.

When a drop of methylene blue is placed on filter paper the water spreads while the dye remains *in situ*, because it is adsorbed by the filter paper which is negatively charged. When eosin is applied both water and dye spread together. This is a ready test for basic v. acidic dyes. Some acidic dyes, namely, those which are highly colloidal (have large molecules or particles), fail to keep up with the water in diffusion but do not form such a sharply defined spot as do basic dyes.

Correspondingly it is relatively easy to wash out an acidic stain from filter paper, whereas a basic one is strongly held. This is not to say that there is no adsorption at all of acidic dyes by filter paper. When we compare the concentration of stain in the paper with that in the solution we usually find that there is an excess in the paper, and the washing out of such a stain is not so

easy as to indicate that the dye is merely in solution in the pores. There is thus some mechanical adsorption in spite of electrostatic repulsion. This is shown strikingly when ions are adsorbed until they not only neutralize but reverse the charge on a surface. Gelatin illustrates this better than filter paper.

The presence of H-ions or polyvalent cations, for example, Al or La, causes gelatin to stain with acidic dyes and not with basic dyes—the reverse of what happens in water or an alkaline solution. Substances which alter their charge according to the concentration of acid or alkali to which they are exposed are termed **amphoteric.** The intermediate state of acidity or H-ion concentration at which they bear no charge is called their **isoelectric point.**

The charge at a surface is due to separation of ions in either of two ways. Assuming that the substance itself is insoluble, it may nevertheless dissociate, yielding an ion which is soluble and passes into the medium, leaving the surface charged. Alternatively it may take up one kind of ion from the solution in preference to the other—cations in preference to anions or vice versa—thus imparting a charge to the surface. The fact that all chemically indifferent substances (oil, air, carbon, etc.) are negative to water is probably due to the greater capillary activity of OH as compared with H-ions. In both cases, however, we must picture not a single but a double layer of ions—one held either by mechanical adsorption or else by its cohesion to the neutral mass to which it belongs, and the other layer of ions of opposite sign, soluble, but prevented from diffusing very far by the electrostatic attraction of the first layer (Fig. 26).

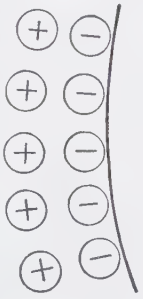

Fig. 26.

Any individual ion, however, may escape from the outer or aqueous layer if another of the same sign takes its place. Electrical adsorption is supposed to consist mainly of an exchange of ions in this outer layer: but if the exchange is to neutralize in any measure the charge on the adsorbent, the ion which is substituted must approach closer thereto than that which is displaced and must adhere to the adsorbing surface instead of remaining in solution. Since various causes may make it do this it is hard to separate electrical from mechanical or from chemical adsorption,

and better on the whole to speak of adsorption of ions without assuming the mechanism.

The fact that strongly adsorbable ions may reverse electric charge indicates that ions may sometimes be adsorbed in spite of and not because of the charge they bear. Further discussion of the ionic double layer is reserved until we come to deal with its electrical effects.

CHAPTER VI

ADSORPTION IN CELLS

The principal effects of adsorption in cells may be classed under the following heads:

(1) *Lowering of surface tension.*—Mechanical adsorption involves lowering of surface tension, the possible effects of which on protoplasmic form, movement, adhesion, etc., have already been considered.

(2) *Change of electric charge and thus of apparent surface tension.*—Adsorption of ions usually tends to reduce surface charge and therefore to raise the apparent surface tension with resulting greater tendency to minimal area and other attendant changes. The remarkable intracellular transformations in *Spirogyra* under the action of Ba^{++} and other cations are probably a case in point. Since the charge on protoplasmic surfaces is generally negative, cations are the active ions. Other results of reduction of charge, such as greater tendency to adhesion and agglutination, are discussed below as well as under colloids.

(3) *Changes in colloidal state.*—The above named changes acting on colloidal particles may produce indirect effects of great importance in biology. These are dealt with in the chapters on colloids.

(4) *Formation of films and membranes*; conversely their destruction by capillary active solutions (see below).

(5) *Local accumulation and binding of substance*; displacement of the substances in capillary active solutions (see below).

(6) *Regulation of chemical action.* (*a*) Inhibition by removal of reactant from solution; (*b*) promotion (catalysis) by bringing reactants suitably together at an adsorbing surface (see below).

I. MECHANICAL ADSORPTION IN CELLS

1. Protoplasmic Membranes: Origin and Alteration. (*a*) *The Plasma Membrane.*—We have already indicated briefly in dealing

with cell structure and shall do so more fully in connection with permeability that there exists a differentiated layer on the surface of cells, a layer which prevents the more aqueous internal protoplasm from mixing with water, and which is less permeable than the interior to water-soluble substances. The properties of the external layer are such that we must assume a greater concentration of organic substance, and one theory of its origin regards it simply as a **concentration membrane** of the type that arises by adsorption at the boundary of two immiscible liquids of a third (colloidal) substance which lowers the interfacial tension, a so-called " haptogen membrane." Certain features of its origin might be regarded as favouring the adsorption theory. There is a time factor in its formation—microdissection shows that a rapid and extensive rupture of the film may result in dispersion of the protoplasm in the medium before a new film has time to form (Fig. 1*b*). Also there is a viscosity factor—low protoplasmic viscosity favours its formation—which at least points to a diffusion rather than a coagulation process as determining the time factor.

There are difficulties, however, in adopting so simple a theory as that of a concentration membrane. If the protoplasm is water-miscible until the film has formed, this implies that when fresh protoplasm is exposed there is no definite interface for the film to be deposited upon. On a colloidal scale it is equivalent to bringing an emulsion of oil in water in contact with water. The theory of a **coagulation membrane,** such as forms when albumin comes in contact with water, avoids this difficulty but meets with the objection that the protoplasmic membrane is a liquid film and not a solid coagulum like precipitated protein. The fluidity, water immiscibility, and—as we shall see later—solution affinities of the membrane all point to its being largely of a lipoid nature. This has suggested the theory that the membrane is a kind of " myelin " formation. A lipoidal substance, lecithin, which is known to be widely present in cells, has, as we have seen, the peculiar property of extending as thin films in a watery medium without any other phase to spread upon. The theory (Leathes') that the plasma membrane forms as films of lecithin do has therefore much to commend it. This itself is an adsorption phenomenon of a kind, and, once the film has formed—by whatever method—it furnishes the required interface for further adsorption; thus after all it is probable that although the plasma membrane may not be

a simple concentration membrane it is largely a product of adsorption.

But just as films may be formed by surface concentration, so also may they be altered or destroyed by the adsorption of other capillary active substances, as in the destruction of a soap foam by alcohol. As regards the plasma membrane, proof of its alteration by adsorption is more direct than of its production thereby. We shall see later that *all substances which notably decrease its permeability are highly adsorbable*, for example, narcotics (see below) and polyvalent cations. In higher concentrations these and other substances produce the opposite effect on permeability accompanied by injury to the cell. Quantitatively it is found in many cases, especially among chemically allied compounds, that equally capillary active solutions are equally toxic. Thus among the alcohols as well as certain other homologous series Traube's rule for adsorption holds also for toxicity. In accordance with the rule each successive member of the series is about three times as capillary active as its predecessor and three times as toxic—that is to say that one-third of the concentration has the same results. The capillary activity is measured at a water-air surface but, as was pointed out, there is commonly a parallelism at least within a homologous series between the adsorbability at air and other interfaces, although in passing from one series to another we find discrepancy due to specific attraction of the adsorbent. The same is true of relative toxicity.

Symbasis between toxic action and capillary activity is thus strong proof that the substances which show it act through adsorption. We infer from the accessibility of the cell surface and from the results on permeability, etc., that the plasma membrane has a prominent share in this adsorption. How it is affected will be discussed later.

A visible example of the effect of a capillary active solute on the permeability and other properties of the plasma membrane is shown in hemolysis by saponin. Not only does the hemoglobin (which is most generally believed to be dissolved in the fluid cell interior) escape through the envelope, but the shape and adhesive properties of the cells are also changed.

(*b*) *Intracellular Membranes.*—Membranes which form in the interior of cells, though similar in type to the plasma membrane, differ considerably from it and among themselves. Thus the

lining of the vacuole is much more resistant to injury than the rest of the cell. It may still act as a semi-permeable membrane for days and even weeks after the cell as a whole has been killed by iodine or by strong plasmolysis (de Vries, Lloyd). On the adsorption theory this is not unexpected because both phases at an interface may contribute to adsorption and the contribution of the vacuole containing as it does much colloidal matter dissolved in an aqueous medium is bound to be considerable.

2. Cell Walls and Coverings.—The commonest kind of protective covering to a living protoplast is that of an organism such as *Difflugia* which is invested by grains of sand picked up from the surrounding medium. Whether we call it adhesion or adsorption, it is simply a question of lowering surface energy. Some Rhizopods become invested by one kind of particle, others by another, but the selectivity displayed by different organisms is no greater than the selectivity of adsorption.

In other organisms (*Quadrula*) particles of $CaCO_3$ or other material which form within the cell become concentrated at the surface—no doubt for a similar simple reason. More commonly the investing substance, mineral or organic, is excreted in a finer (colloidal or molecular) state of subdivision, and assumes various forms, crystalline or amorphous, after reaching the surface; but there is no less reason to suppose that adsorption plays its part. Thickened membranes as well as monomolecular layers may be formed through this agency. For example, the protein film on soup or boiled milk is made up of very many layers of protein molecules. Each one when adsorbed becomes chemically changed and offers a surface for the adsorption of a new layer.

One must not suppose that adsorption alone is adequate to explain the origin of the cell wall any more than of the plasma membrane. The more one studies the walls of plant cells the more does one realize the complexity of the structure of the cell and of the organization that produces it.

3. The Action of Narcotics may be considered here since the bulk of opinion tends to regard its most characteristic features at least as a membrane effect.

There is a group of organic substances which tend temporarily to abolish the irritability and automatic activity characteristic of life. To these the name narcotics (making numb) or anesthetics (abolishing feeling) is applied on account of the particular action

on the nervous system, but all kinds of cells are affected by them as regards irritability. The same physiological effects are also produced by other agencies, for instance, by certain inorganic salts (of Mg, K, etc.), by removal of electrolytes, by cold, by electrical polarization, etc.

What interests us is the mechanism of their action. There is no doubt that a close parallel exists between narcotic activity and lipoid solubility, both among the organic and inorganic agents (Overton; Meyer) but as we have seen this may equally well indicate adsorption (Traube; Warburg).

Actually in a system such as the cell where the physiologically active lipoids exist either in molecular films or in colloidal dispersion the distinction between solution and adsorption by them has little meaning. On the whole the obedience to Traube's rule favours adsorption. Moreover Warburg has shown that narcotics as regards some of their activities (in depressing the oxidation which can go on for a time in dead cells) are just as effective after extraction of lipoid from the cells.

As we shall see later, narcotics inhibit many kinds of physiological process—always apparently through adsorption—but the most characteristic feature of narcosis—insensibility to stimulation—is produced by lower concentration than is required for any other inhibition (except stoppage of photosynthesis). According to R. S. Lillie, the condition opposite to narcosis, heightened excitability, is always accompanied by increased permeability. Narcotics counteract this condition both as regards excitability and permeability. Acting alone they appear to reduce permeability below normal. Although organic narcotics penetrate freely, a salt $MgCl_2$ which penetrates very slowly is also a powerful narcotic. Hence it is assumed that a certain feature of narcotic action, and that the most typical, is localized in the plasma membrane. How adsorption of narcotics can decrease permeability is a question that must be left over until the complex mechanism of permeability has been discussed.

4. Adsorption in Relation to Chemical Reactions.—Two quite opposite effects may be produced in different cases by adding a colloid to a chemical mixture. A reaction which would take place in absence of the colloid may be slowed down or prevented; or contrarily a reaction may be induced or accelerated. The inhibitory effect is the result of the removal by adsorption of one (or

both) reagents from the *venue* of their interaction, that is, the solution. The acceleratory or catalytic effect is the result of bringing the reactants together at the adsorbing surface when the reaction can go on there. In large degree the change in velocity of reaction whether in the solution or at the surface may be explained by changes in concentration, but there is no doubt that the factor of molecular orientation at surfaces in bringing the reacting parts of molecules together, or keeping them apart, is also of great importance, especially in protoplasm.

(*a*) *Inhibition.*—An example with *inorganic* reagents is the inhibition of the Prussian blue reaction by a positively charged colloid, for example, Al_2O_3. If a suitable concentration of $K_4Fe(CN)_6$, followed by $FeCl_3$, is added to water the blue reaction appears instantaneously [$M/10000$ $K_4Fe(CN)_6$ and M 1000 $FeCl_3$—as diluted—are suitable concentrations]. If added in the same way to an Al_2O_3 solution instead of water there is no colouration save gradual and slight after a long time. $Fe(CN)''''_6$ is adsorbed by the positive colloid, Fe''' is not. The reaction in the solution is inhibited by the lowering of the $Fe(CN)_6$ concentration.

An *organic example* of inhibition is the deactivation of any enzyme. Rennet shaken with charcoal fails to cause coagulation of milk. It is adsorbed by the charcoal. A *biological example* is the deactivation of tannin in the ripening of fruit. Green bananas and many other unripe fruits have a highly astringent taste due to tannin, but lose this astringency during ripening without losing their tannin (Lloyd). In the unripe fruit the tannin is in solution in the sap of certain cells. In the ripe fruit it is adsorbed by a colloidal mass that develops in these cells. It is still able to react slowly with ferric chloride on the colloidal surfaces (producing under the microscope a homogeneous-looking blue mass instead of a precipitate as appears in solution) but it is not able to go into solution and affect the organs of taste, etc.

(*b*) *Catalysis.*—Insoluble Inorganic Catalysts such as platinum black and carbon are powerful adsorbents, and it is at their surface that reactions are catalyzed. Those *organic* catalysts, termed enzymes, or ferments, which so far have been produced only by living cells, also act partly through adsorption. Organic catalysts have been produced artificially but it is not the custom to call these enzymes. The proofs that enzymes act at their surfaces are many. They are colloidal; indeed they may act in

solvents in which they are not even colloidally soluble but only coarsely suspended; the relation between concentration of substrate (reagent) or of enzyme and rate of reaction resembles an adsorption curve (complicated by relative mutual adsorption by enzyme of substrate, or by substrate of enzyme); the reaction is promoted or respectively inhibited by ions ($H^{\cdot}$, $Ca^{\cdot\cdot}$, etc.) according as they increase or decrease electrical adsorption between enzyme and substrate; and it is inhibited by capillary active substances in proportion as they are adsorbed.

While many enzymes are fully active *in vitro*, others obtain their full efficiency only in the cell, for instance, zymase which causes alcoholic fermentation. It has been suggested that a further adsorption of enzyme and substrate at structural surfaces in the cell is the distinguishing factor, but the possibilities are many. Whether other biological processes such as respiration and assimilation of CO_2 which are dependent on cellular structure are catalyzed through the intervention of enzymes which have not been isolated by the living substance it is impossible to say, but the evidence shows that in these too adsorption takes place. The indications of this are: (1) the fact that they are inhibited by capillary active substances (Traube's rule being obeyed) and (2) that they may be imitated to some extent by physical models involving adsorption. The following table (Warburg) illustrates the first of these proofs.

Inhibition of Respiration of Living Bird Blood Cells to 50 Per Cent

	Molar Concentration	Per Cent Lowering Surface Tension of Water
Methyl Alcohol	5.0	31
Ethyl	1.6	28
Propyl	0.8	35
Butyl	0.15	28
Amyl	0.045	28

Respiration model.—Warburg discovered that certain foodstuffs, especially amino acids are oxidized at ordinary temperatures when adsorbed by blood charcoal. As with respiration this

reaction is inhibited by capillary active substances. In any homologous series Traube's rule is followed, but Warburg went further and linked one series with another by comparing the inhibiting action with the amount adsorbed at a charcoal surface and still better with the calculated area covered assuming a monomolecular layer. He finds that oxidation is reduced in proportion to the area covered. The poisoning action of narcotics on respiration, etc., is thus imitated by their action on charcoal.

But still another type of poisoning is simulated in these experiments. It was found that only carbon with traces of heavy metals, particularly Fe, catalyzed the oxidation except in slight degree. This is only one example of a general rule that oxidation-reduction reactions are facilitated by traces of a heavy metal such as Fe, Cu, Mn, Va, which is capable of existing in two states of oxidation: $Fe^{\cdot\cdot}$ and $Fe^{\cdot\cdot\cdot}$. Oxidase enzymes probably contain traces of Fe or Mn; hemoglobin contains Fe; chlorophyll contains Mg and requires Fe for its production.

Now HCN though only weakly adsorbed may inhibit the oxidation on charcoal as completely as the most capillary active substance. It also inhibits biological oxidation. It is suggested that the reason is that HCN combines with the Fe or other heavy metal which, attached to the adsorbing surface, is the real chemical catalyst. This illustrates the fact that the catalytic activity of a surface, depending on its chemical as well as physical nature, may be more or less specific.

5. Adsorption and Immunity from Disease.—Adsorption plays an important part in the various reactions by which the animal body protects itself against the attack of pathogenic bacteria. The resistance is effected by the phagocytic destruction of the bacteria and the detoxification of their injurious products. In certain cases substances which facilitate this resistance are produced in the blood as a result of the infection itself—or its equivalent—giving rise to subsequent immunity. These substances are classed as **opsonins,** those which facilitate ingestion of the bacteria by leucocytes; **agglutinins,** which cause agglutination or sticking together of bacteria and thus also facilitate ingestion; and **antitoxins,** which neutralize the bacterial poisons or toxins. The first two are adsorbed by the bacterial surface while toxin and antitoxin adsorb one another.[1]

[1] See Oertel, General Pathology; Dreyer and Douglas, Proc. Roy. Soc. B. *82* : 185 (1910); Ledingham, Journ. of Hygiene *12* : 320, (1912).

A proof of adsorption in these cases is the fact that the relation between concentration and amount taken up obeys the adsorption isotherm $a = kc^{\frac{1}{n}}$; whereas the inference that adsorption of opsonin and agglutinin takes place at the external surface of the bacterial cells rather than inside them, follows from the speed with which equilibrium is attained and from the changes in surface energy which result. The exact nature of these surface changes is somewhat obscure. In the case of opsonins the interfacial tension between bacterium and phagocyte is presumably reduced—or in other words adhesion is increased. In the case of agglutinins the surface of the bacteria is changed in such a way (sensitized) that the Ca and other ions present in the blood plasma flocculate them. This phenomenon of " sensitization " as it is called is shown with respect to other foreign substances in the blood besides bacteria and is known to the pathologist as **anaphylaxis.** Sensitization is well known in physical colloids and will more conveniently be discussed under that head, as will also the mutual adsorption of toxin and antitoxin.

A feature of these and some other biological adsorptions is the specificity in many cases of the relation between adsorbent and adsorbate. Typhus agglutinin is taken up strongly only by typhus bacteria, diphtheria toxin by diphtheria antitoxin; enzymes are highly selective in their action; and so on. Theoretically, however, according to Freundlich there is no reason why adsorption should not be specific, and, although there is a gap in the theory because it has not been possible so far to obtain it with substances manufactured in a test-tube, the condition is foreshadowed, for instance, in the *complete* instead of partial displacement from charcoal of a more weakly by a more strongly adsorbable amino acid—indeed more of the latter is taken up from the mixture than from the pure solution. Perhaps a further study of adsorption by proteins, etc., will reveal phenomena which correspond to the specific adsorption of toxins, agglutinins, etc.

II. ELECTRICAL ADSORPTION IN CELLS

1. Electric Charge on Protoplasmic Surfaces.—Protoplasmic, like other surfaces, if they bear an electric charge, should follow the rules of electrical or polar adsorption in the binding of ions. The bulk of the solid matter of protoplasm is amphoteric, that is,

capable according to conditions of assuming either a + or − charge, or, third, no charge. This applies to proteins, amino acids, lipoids, nucleic acid, etc. The chief of these are the proteins, and a word may be said as to how the charge on these originates. Like the amino acids of which they are composed, their formula may be represented as HROH with the capacity to dissociate both H^+ and OH^-. $H^+ + ROH^- \rightleftharpoons HROH \leftrightharpoons HR^+ + OH^-$. Even in solution the protein part R is of sufficiently large size to show properties of surface and adsorption. We see that it may be negatively charged if more H than OH ions are dissociated and positively charged if the reverse is the case. The relative dissociation of H^+ and OH^- depends partly on the inherent tendency of the particular compound and partly on the respective concentration of these ions in solution, that is, on the acidity or alkalinity of the solution. Thus we find that in alkali proteins are negative and migrate to the anode, whereas in acid they are positive and migrate to the cathode. At a certain reaction of the medium—in the case of proteins on the acid side of neutrality —H- and OH-ions will be equally dissociated and the particles will have no charge. This is called the **isoelectric point** with respect to *H-ion concentration*. Substances which behave in this way are said to be amphoteric electrolytes or ampholytes.

On the alkaline side of their isoelectric point proteins tend to attract cations and on the acid side anions. The combination is chemical in the case of acids and alkalis but with salts it is generally ascribed to adsorption. The adsorption of ions in turn influences the charge on the proteins, so that strongly adsorbed ions of any kind may markedly shift the equilibrium and isoelectric point with respect to H and OH ions.

The charge on cells and on particles within the cell, like that on any small body, is best detected and measured by **electrophoresis** (or kataphoresis), that is, transport by an electric current (see under Colloids). Living cells (blood cells, bacteria, algae, etc.) are usually negatively charged. The charge varies with the electrolytic environment in the same way as with lifeless suspensions. Polyvalent ions and other colloidally active (adsorbable) ions reverse it. The H-ion reaction at which human red blood cells are without charge, that is, the isoelectric point, in absence of polyvalent ions, is about *p*H 4.6. The position of this point shows a certain specificity, varying with the species of animal. In

the case of bacteria the electrokinetic potential varies even with different strains of a species. An extremely suggestive discovery is the relation of charge to virulence among strains of *pneumococcus*. The greater the interfacial potential of the strain the greater its virulence—possibly because it has less tendency to adhere to negatively charged phagocytes.

The charge on particles within the cell is more difficult to determine electrophoretically and the results are contradictory. Recent determinations (Taylor, Proc. Soc. Exp. Biol. Med. **22**: 533, 1925), with the aid of microelectrodes inserted into the protoplasm of a slime mould, give the result that microscopically visible particles are negative while of the ultramicroscopic particles some are negative, some positive and some neutral.

The effects of the adsorption of ions by protoplasm are best illustrated by the colloidal changes which result and will be noted when we come to deal with that subject. At present we shall confine ourselves to the adsorption of dyes, which can be followed simply by staining effects. It may be noted however that colourless organic cations, as in alkaloids, have also a powerful physiological action which may possibly be due to their adsorbability. In a heterogeneous system like the cell, different surfaces naturally have different charges because the various proteins and their compounds have different isoelectric points; moreover, if acidity varies locally, one and the same protein may be + in one part and − in another. Also by varying the reaction of the medium the charges on the proteins, etc., may be changed. All these possibilities are illustrated in the staining of both living cells and dead cells.

2. Post Mortem Staining.—When dead cells are stained with a mixture of acidic and basic dye of contrasting colour, say methyl blue (acidic) and safranin (basic) or eosin (acidic) and methylene blue (basic) and are then washed in solutions of different H-ion concentration, the macroscopic behaviour is the same as with gelatin or ablumin. The basic dye is washed out in a decidedly acid medium and the acidic dye in a neutral or alkaline medium; the cells become blue with (acidic) toluidin blue in the first case and red with safranin in the second, whereas in an intermediate slightly acid zone (around N/10,000,000 H-ion concentration) the purplish colour of the combined dyes persists. Microscopic examination shows that in the solutions in which both dyes are held,

certain parts of the cell may be coloured by only one dye and other parts by the other. On the theory of electrical adsorption this should tell us something as to the isoelectric point of these various constituents. To some extent, however, this varies with the method of killing or fixing the cells. Such common fixatives as chromic acid and mercuric chloride combine more or less irreversibly with the organic components, altering their isoelectric points to varying degrees. Less change in this respect will be induced by non-electrolytes or by heat as killing agents—though these are but poorly adapted to preserving structure.

The long standing histological classification of stains as " nuclear " and " plasmatic " (staining nucleus and cytoplasm respectively) is found to agree with the chemical classification into basic and acidic. Actually, however, there is a much sharper distinction between the basophil and acidophil tendencies of certain elements of both nucleus and cytoplasm than between these structures as a whole. In the resting nucleus the nucleolus is more basophil than the chromatin (which may stain with either kind of dye) whereas the chromosomes of dividing nuclei are apparently more basophil than the chromatin or spireme from which they develop. (Probably the proportion of nucleic acid to protein in the nucleo-protein substance of which chromatin mainly consists, increases during mitosis.) Other elements of the fixed nucleus are usually acidophil, namely, " linin " and spindle threads. Similarly, although the bulk of the cytoplasm may be acidophil, basophil granules are usually present in greater or less degree, varying with the kind of cell and its stage of development.

The results obtained are not entirely determined by electrical adsorption; mechanical adsorption is powerful in dyes and more so in some than in others. Thus a strongly adsorbable acidic dye like eosin or a basic one like safranin is often adsorbed in spite of moderate electrical repulsion, especially by strongly staining structures like the nucleolus and chromosomes.

Cell walls. Differential staining of cell walls in sections of plant tissue is obtained by using a basic and an acidic dye of contrasting colour. If a section is first stained with a basic dye and then for a suitable priod with an acidic one, the cellulose walls lose the basic and take on the acidic stain, although lignified walls still retain the colour of the former. (Lignin in virtue of the acid

(phenolic) group in its molecule is much more strongly negative in water than cellulose is.)

3. Vital Staining.—The staining of living cells is limited by their permeability to the dye as well as by their tendency to store it up in the cell interior whether by adsorption or otherwise. Basic dyes nearly always penetrate freely; acidic dyes only do so in some kinds of cells. Microinjection and other modes of artificial penetration, however, enable us to know something of the staining ability of acidic dyes in those cells which are impermeable to them.

The results with both classes of dye differ markedly from post mortem staining. The ground substance of the cytoplasm and also of the plastids in a healthy cell and under normal condition is practically unstainable by most dyes, basic or acidic. The dyes are soluble enough in the cytoplasmic matrix, as shown clearly by microinjection. But they do not concentrate there in equilibrium with a dilute solution outside, that is, they are not notably adsorbed. It would therefore appear that cytoplasm, like serum proteins, is non-ionic. In serum it has been shown that this is due to combination of proteins (Hardy).

The effect of penetrating alkali and acid on vital staining of protoplasm favours the hypothesis of a non-ionic or isoelectric condition. As with proteins in general, acid promotes staining of the cytoplasmic matrix and plastids by acidic dyes whereas alkali sometimes allows a faint diffuse stain with basic dyes. We must be cautious, however, in drawing conclusions from effects of acid and alkali because these alter the condition of the dye and of the permeability of the plasma membrane as well as the charge on the cytoplasmic colloids.

The more lipoid soluble dyes are said by Mollendorf to stain the ground substance without any assisting factor. *Most dyes,* however, if they penetrate, *are stored away in granules or vacuoles* (or for all we know are excreted again in some way) and only under abnormal conditions reach a visible concentration in the clear cytoplasm.

Granules.—Basic dyes nearly always stain granules and droplets in the cytoplasm. To a large extent these pre-exist the staining, although the latter, probably through reduction of charge, induces their coagulation into larger aggregates. It is evident, however, that the stained particles also grow in size; whether

through deposition of fresh material from the ground substance, or through swelling, is uncertain. Just as the more apolar or lipoid soluble a basic dye, the more it tends to stay in suspension (though possibly adsorbed) in the ground substance, so the more polar it is the greater the tendency to precipitate the adsorbing particles and leave the cytoplasm clear. This is duplicated in the relative tendency to precipitate acid (negative) colloids such as acidic dyes (Mollendorf).

It would appear from the distribution of vital stain in the cytoplasm that negatively charged colloids, that is, acid substances like tannic acid, etc., of which the anion is colloidal, are mainly concentrated in granules or small vacuoles—the two are difficult to separate. Although most granular staining is of such a general character that simple electrical adsorption is adequate to explain it, certain types of granule show a particular attraction for certain dyes which must result from a more specific affinity than merely that of opposite charge. Thus *mitochondria*, which are said to be particularly rich in lecithin, are especially stained by Janus Green and chemically allied (lipoid soluble) stains. Present knowledge of specific vital staining is too meagre and indefinite however for us to dwell upon it. When acidic dyes produce granular staining in living cells the granules are said always to arise *de novo* and to resemble small vacuoles rather than solid particles.

Vacuoles.—Vacuoles including the large central sap vacuole of plant cells often stain with basic and less commonly with acidic dyes. If the sap contains visible suspended granules these usually behave as do those in the cytoplasm but the fluid medium also may accumulate stain. Though the stain may appear to be a homogeneous solution, the evidence goes to show that basic dyes at least are stored by adsorption or some form of combination with " dissolved " colloidal material. Thus dye and colloid frequently precipitate together; or the colloid before or after staining may be precipitated by other agents, for example, alkaloids or weak alkalis. The colloid is probably a *complex*, varying with the type of cell. In many plant cells it is rich in tannic acid. Other cells have other acids.

The stainable colloid has been shown in many cases to behave as if it were amphoteric, losing its attraction for basic dyes and acquiring an attraction for acidic dyes when the acidity becomes sufficiently high. (The H-ion concentration of vacuoles unlike

that of the cytoplasm is easily modified by penetrating acid and alkali.)

All these facts point to basic dyes being adsorbed—or at least bound in some way to colloid particles—both in the cytoplasm and in vacuoles. The failure of basic indicator stains, even when apparently homogeneously dissolved in the vacuole sap, to function properly as indicators or to combine with acidic dyes as they do *in vitro*, is further proof of such binding.

The evidence is not so clear as regards acidic dyes. In certain cells they may accumulate to a high degree in the cell sap and remain there however long the cells are washed; yet they still react with basic dyes which enter later, or—if indicators—to acid and alkali. In fact acidic dyes stored in the sap behave as do the natural pigments of coloured cells.

Nucleus.—Staining of the nucleus is commonly taken as an indication of injury or death of the cell, but examples of vital staining, whether in absolutely normal cells or not, are common. The nucleus, and especially the nucleolus, stains more readily than the cytoplasm in life as in death. Here again staining with acidic dyes is brought about or aided by acid, but alkali seems to reduce the staining capacity with any kind of dye. It swells the chromatin material or chromosomes. The body of the nucleus may be homogenously stained (with an acidic dye at least) or, if the chromatin is segregated, there may be differential staining. The following results have been recorded of vital staining with neutral violet (neutral red 2 parts, new blue 1 part) in 0.025 per cent solution, with an acid reaction *p*H 4.38 (Kuwada and Sakamura).[2]

In the meristem tissues of plants the chromatin of resting nuclei is blue (+) and the nucleolus red (−); but in mature internodal cells, where cell division has ceased, the chromatin is red (−). In the early stages of mitosis the spireme and chromsomes are still blue (+) but in all cases the chromosomes become temporarily red (−) during the metaphasic stages when the nuclear membrane disappears. These results agree closely with those of post mortem staining, which fact supports the view that it is determined simply by adsorption. The cytoplasm, it may be added, is blue to violet under the same treatment as above, so that a greater con-

[2] Kuwada and Sakamura, Protoplasma, I, 239, 1926. Since the *p*H of the dye solution was 4.38, complete normality of the cells may be doubted. Kuwada and Sakamura, Protoplasma *3* : 531, 1928.

trast of basophily v. acidophily is shown by the different components of the nucleus and by the same component at different stages than between nucleus and cytoplasm as a whole.

Cell wall.—The staining of cell walls is naturally much the same in living as in dead cells. In the former, however, cellulose walls appear to be slightly less negative. The affinity for basic dyes is somewhat less and a lower concentration of Ca^{++} or other polyvalent cations changes it to acidophil.

Mechanical adsorption in vital staining.—In vital colouring of nucleus, cytoplasm and plastids and cell wall, as in staining in general, the eosin group of dyes accumulate more readily than most other acid dyes, another point in favour of the view that vital staining is an adsorption phenomenon, but also showing that it is mechanical as well as electrical.

The Importance of Solutions in Life

Water is the continuum between an organism and its environment. All cells, at least when active, are bathed in water and are themselves largely compounded of it. In neither case is the water pure, but contains dissolved substances. The properties of solutions in general and of the particular solutions which can maintain life is therefore a subject of great importance to the physiologist. Also the behaviours of cells in allowing some substances to pass through them and not others, calls for a study of the properties of membranes with respect to solutions. The principal phenomena to consider are diffusion, osmotic pressure and the conductance of electricity.

CHAPTER VII

DIFFUSION AND OSMOSIS IN PHYSICAL SYSTEMS

I. DIFFUSION

Diffusion is particulate or molecular—as opposed to mass—movement from higher to lower concentration. It is most simply displayed by gases but substances in solution resemble gases in their kinetic behaviour. In both, the molecules are in active movement. Figure 27 represents diagrammatically the diffusion of dissolved particles from a crystal undergoing solution. The concentration diminishes steadily from the source outward and fades out toward the diffusion front, as can be seen experimentally if a coloured crystal is used.

FIG. 27.—(Holman and Robbins.)

Diffusion in gases is explained by the greater number of impacts which the particles encounter on the more concentrated side. The mean free path is greater toward the more dilute side. The same applies in a solution although the attraction of the solvent is an additional factor. Whatever the mechanism, diffusion is a manifestation of the general law of the tendency to **equipartition of energy.** All kinds and sizes of particles possess the same kinetic energy ($\frac{1}{2}\ mv^2$, where m = mass, v = velocity) and each kind tends toward uniform distribution in the field of diffusion. In solution as compared with gases, however, the actual rate of diffusion is of an utterly inferior order of magnitude on account of the friction of the medium. In other words, collision with solvent molecules vastly reduces the mean free path.

Let us consider the various *factors on which the rate of diffusion depends.*

(1) *Size of diffusing particle.*—Since $\frac{1}{2}\ mv^2$ is constant the average velocity of a particle varies inversely as the square root of its mass ($v \propto \frac{1}{\sqrt{m}}$). With gases this relation holds for the rate of diffusion. It also holds approximately in the case of small molecules in solution. For large molecules and colloidal particles the relation is different. The rate of diffusion tends rather to vary inversely as the radius of the particle. The theoretical formula for the frictional resistance to the movement of a sphere which is large compared with the molecules of the liquid is $6\ \pi\eta r$ where η is the coefficient of viscosity of the liquid and r the radius of the particle.

In practice, in the case of crystalloids the molecular weight is usually known. Variation of the actual from the calculated rate of diffusion is taken as an index of the hydration of the particle. With colloids it is more usual to discover the size of the particle from the observed rate of diffusion.

(2) *Permeability of the medium.*—In addition to the friction or viscosity (η) of the medium which determines the rate of movement of individual particles, diffusion is regulated by the *dissolving power* of the medium which regulates the number of particles that can, as it were, move abreast through it. The relative rate at which different substances pass through a homogeneous membrane is determined more powerfully by their solubility in it than by any other factor. The rate varies directly as the solubility.

(3) *Temperature.*—The inherent velocity of a particle is proportional to the absolute temperature, which would mean quite unimportant variation within the temperature range of life. The effect of temperature on diffusion, however, is enhanced by its effect on hydration of particles and in reducing the viscosity of the medium. Thus the rate of diffusion of NaCl in water at 50° C. is four times that at 0° C.

(4) *Gradient of concentration.*—Rate of diffusion varies directly as the gradient of concentration of the diffusing substance, that is, as the difference in concentration between two points divided by the distance apart. If we take the simple case of diffusion across a membrane when the concentration is maintained constant on each side, the formula may be written

$$\frac{s}{t} = Da\frac{C_1 - C_2}{x}$$

Where s = the amount of substance passing through a plane of area a in time t, C_1 and C_2 being the concentration at starting point and end point respectively, and x the thickness of the membrane. The constant D, called the coefficient of diffusion, is thus independent of concentration (Fig. 28).

Since ordinarily the very process of diffusion tends to reduce the gradient of concentration so that the gradient varies from point to point as well as from moment to moment (Fig. 29), the equation must be expressed differentially:

$$\frac{ds}{dt} = Da\frac{dc}{dx}$$

The relation between rate of diffusion and concentration gradient is called Fick's law of diffusion, but the expression holds for every

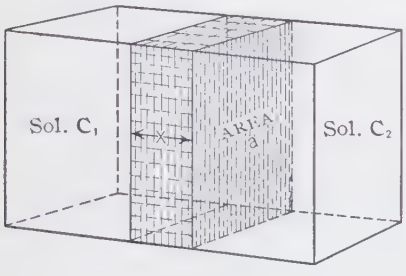

Fig. 28.

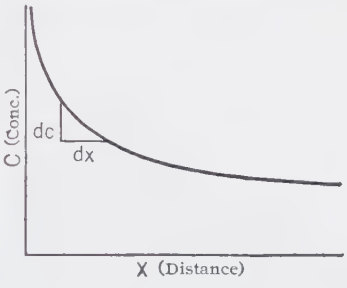

Fig. 29.

kind of diffusion, for instance, of heat, electricity, etc., as well as of material particles.

II. OSMOSIS AND OSMOTIC PRESSURE

1. Manifestations of Osmotic Pressure.—The special name **osmosis** is applied to diffusion through a membrane. When a third phase (membrane) is interposed between two media complication may arise owing to the membrane's being unequally permeable to the constituents of the media. When permeable to solvent but not to solute the membrane is said to be semipermeable to the solution in question. The momentum of the impinging solute molecules creates pressure on the constraining barrier just as gas exerts pressure on its container. In solutions this pressure is termed **osmotic pressure.** It expresses itself in various ways

besides exerting pressure on a semipermeable membrane. Just as diffusion in gases is due to the driving force of pressure so is diffusion in liquids due to osmotic pressure. Expressed generally, just as a gas tends to expand in proportion to its pressure, so does a solution tend to dilute itself (by diffusion and osmosis) or to resist concentration (for example, through loss of water by evaporation or freezing) in proportion to its osmotic pressure.

2. Direct Measurement of Osmotic Pressure.—Osmotic pressure is usually measured by its indirect effects. Direct measurement on principles analogous to the measurement of gas pressure is simple enough in theory as illustrated in Fig. 30. The solution (A) is divided from water (B) by a membrane (m) permeable to water but not to the solute in A, that is, semipermeable. A semi-permeable membrane is necessary to allow solvent to enter or escape, otherwise of course a solution cannot change its volume as can a gas. As it is, the solution expands as water enters with a force or pressure per unit area which can be measured by the tightly fitting piston C. That pressure applied to the piston which just prevents expansion is equal to the osmotic pressure.

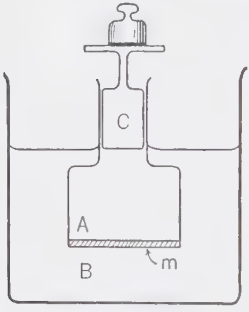

FIG. 30.

In actual practice the measurement involves great technical difficulties owing to the enormous pressures. The best semipermeable membrane is gelatinous copper ferrocyanide, which, to resist pressure, must be deposited in a rigid supporting structure such as porous porcelain. The solution is placed in a closed vessel of this nature with a pressure gauge attached and the vessel is immersed in pure water.

Pfeffer was the first to measure osmotic pressure. He found that it varied with concentration, temperature, and the nature of the solution. The last of these factors was more fully studied by De Vries, who compared the osmotic activity of some 100 different substances, using plant cells as osmometers. In spite of the sources of error in such a method the general agreement of his results with subsequent physical measurements is remarkable. With the various kinds of solution de Vries compared the concentrations

which are just able to produce plasmolysis. Assuming that the protoplasm remains practically impermeable to the solute for the duration of the experiment, such concentrations must be **isosmotic** or **isotonic.** He found that many (organic) substances have the same osmotic pressure for the same molar concentration. Others fall into groups of higher osmotic pressure but similar within the group. The term "isotonic coefficient" as it is now used means the ratio of the osmotic activity of the solution in question to that of cane sugar taken as unity.

3. Laws Applying to Osmotic Pressure (Van't Hoff).—Van't Hoff on the basis of Pfeffer's and de Vries's results showed that the same laws that had been discovered for gas pressure apply to osmotic pressure. At first electrolytes appeared to be discrepant but when their dissociation was discovered by Arrhenius, Van't Hoff's generalization was rendered complete. The gas laws as applied to solutions are:

Boyle's Law: Osmotic pressure varies as molar comcentration.—In the case of electrolytes, since each ion is equivalent osmotically to a molecule, the molar concentration has to be multiplied by a factor i (isotonic coefficient of de Vries) to allow for this. $P \propto C$ or $P \propto iC$.

Charles' or Gay-Lussac's Law: Osmotic pressure varies as absolute temperature (starting from — 273° C. as zero). $P \propto T$.

Avogadro's Law: The osmotic pressure of all equimolar solutions is the same and is equal to the pressure exerted by a gas occupying the same volume.

From the laws of Boyle and Charles, $P \propto iTC$ or $P = RiTC$, where R is a constant for a particular solution. Avogadro's law states that the constant is the same for all solutions and is in fact the " gas constant."

An *ideal solution* would obey these laws absolutely. Dilute solutions (up to $0.1M$) do so very closely. Up to $1M$ Boyle's and Avogadro's laws still hold fairly well if the molecular weight (or fraction thereof) of substance is dissolved in 1000 grams (= 1000 c.c) of water (weight molar) instead of in water sufficient to make a liter of solution (volume molar).

The student of physiology must often use the general formula $P = iRTC$, which summarizes all the above laws. He will do well to remember that the constant $R = 0.082$ when the units employed are: P = atmospheres, T = absolute temperature and

C = mols per liter; and that the osmotic pressure of a molar solution of non-electrolyte is 22.4 atmospheres at 0° C.

In physiological work it is often necessary also to determine osmotic pressure experimentally, the following being some of the ways of doing so.

4. Convenient, Indirect Methods of Determining the Osmotic Pressure of a Solution:

(1) *Lowering of the freezing point.*—A sensitive (Beckman) thermometer is required as the temperature variation is small—only 1.85° C. for a molar solution, or a temperature of − 1° C. for an osmotic pressure of 12 atm. The raising of the boiling point is still less, viz., 0.52° C. for a molar solution.

(2) *Comparison with a solution of known osmotic pressure by means of*: (*a*) *a physical osmometer.* As the semi-permeable membrane a suitably prepared collodion sac may be used (see Brown, Ann. Bot. *36:* p. 433, 1922) or copper ferrocyanide deposited in gelatin (see Laboratory Instructions). (*b*) A *living osmometer.* Usually plant cells, as in the pioneer work of de Vries, but also erythrocytes have been used. This method is limited to solutions which are of sufficient concentration to cause plasmolysis, and are non-toxic and non-penetrating. The methods are described in the Laboratory Instructions.

5. Isotonic Coefficients.—It is often necessary in physiological experiments to make up salt solutions of a required osmotic pressure. To know what concentration of a given electrolyte must be taken, it is necessary to know its isotonic coefficient at approximately the concentration desired. This may usually be obtained from Landoldt and Bornstein's Tables or calculated from the degree of dissociation obtained from Tables. As can easily be verified, $i = 1 + (n - 1)\,\alpha$, where n is the number of ions into which the electrolyte dissociates and α is the fraction dissociated, that is,

$$\alpha = \frac{\text{number of dissociated molecules}}{\text{number of undissociated molecules}}$$

according to the original Arrhenius theory. This is now called the "apparent dissociation" because the real dissociation is much higher—perhaps complete in strong electrolytes—but is partly obscured in measurement by the mutual attraction of oppositely charged ions.

For practical purposes it is convenient to keep note of the approximate values of i that come most into use, namely: 1.8 for monovalent salts with two ions (NaCl, etc.), and 2.4 for divalent with three ($CaCl_2$, etc.) at the concentrations isotonic with average plant cells—equivalent to about M .5 cane sugar or $4\frac{1}{2}$ to 5 atm. osmotic pressure; or 1.74 and 2.33 respectively at the osmotic pressure of mammalian blood. Salts of di- and trivalent ions dissociate less and give lower values of i than those with an equal number of monovalent ions. For $MgSO_4$, $i = 1.10$ at the osmotic pressure of blood.

Example of use of general Gas Law Equation.—Mammalian blood is isotonic with 0.9% NaCl—a so-called "physiological salt solution." Deduce its osmotic pressure.

$$\text{M. W. of NaCl} = 58.5$$
$$0\text{-}9\% = 9 \text{ gms. p. litre} = \frac{9}{58.5} M = .16\ M$$
$$T = \text{body temp. } (37^\circ \text{ C.}) = 310$$
$$P = iCRT$$
$$= 1.74 \times .16 \times .082 \times 310$$
$$= 6.7 \text{ atm.}$$

III. ELECTRICAL CONDUCTIVITY OF SOLUTIONS

Results obtained from conductivity experiments explain facts as to the osmotic pressure and electrical effects of electrolytes, facts which have an important bearing on physiology.

1. Specific and Equivalent Conductivity.—We must distinguish these two things. The first is measured directly and the second — deduced from it — yields information about the solution. Measurement of conductivity (sometimes called conductance) is attained by measurement of its reciprocal, resistance. For this, the ordinary Wheatstone bridge (see p. 121) is used with an alternating current to avoid polarization effects and a telephone instead of a galvanometer as indicator, the latter being insensitive to the alternating current. What is determined is the specific resistance. Just as *specific* gravity means the density (gravity) of a substance as compared with an arbitrary standard, water, so the specific resistance of a solution is its resistance as compared with a hypothetical standard solution which would offer a resistance of 1 ohm if enclosed in a centimeter cube with two opposite faces of the cube acting as electrodes. The numerical value of

the specific resistance of a solution is thus the number of ohms resistance which the solution would offer under like conditions, that is, in a centimeter cube. The specific conductivity is the reciprocal of this figure. It is signified by the Greek letter κ.

The equivalent conductivity (λ) of a solution may be calculated by dividing the specific conductivity by the concentration in gram equivalents. This is equal to saying that it is the conducting power of 1 gram-equivalent of the solute at the concentration of the solution in question. In other words, instead of dividing by the concentration we may multiply by the volume in liters of solution required to hold 1 gram-equivalent. By taking gram-equivalents rather than gram molecules a more just comparison is obtained between the ions in salts of different valency.

With increasing dilution specific conductivity naturally diminishes; the equivalent conductivity on the other hand steadily increases; that is to say, the apparent degree of dissociation increases. In a very dilute solution the equivalent conductivity of a strong electrolyte becomes nearly constant so that it is easy by exterpolation to discover what it would be theoretically at infinite dilution. For weak electrolytes another method has to be employed based on Kohlrausch's law (see below).

2. Calculation of α and i.—The value of λ at any finite dilution is to the value (λ_∞) at infinite dilution as the number of ions in the finite dilution to the total possible number. The degree of dissociation α of the electrolyte is thus calculated from the simple formula $\frac{\lambda}{\lambda_\infty}$

VALUES OF α

N	KCl	CH_3COONa	HCl	CH_3COOH
1.0	0.76	0.53	0.79	0.004
0.5	0.79	0.64	0.85	0.006
0.1	0.86	0.79	0.91	0.013
0.01	0.94	0.91	0.96	0.041
0.001	0.98	0.97	0.98	0.117

As already indicated α is used in calculating isotonic coefficients and so osmotic pressure. $i = 1 + (n - 1)\,\alpha$.

When i calculated from conductivity is compared with i obtained by comparing the osmotic pressure of the electrolyte with that of a non-electrolyte (as by de Vries' plasmolytic method or from the lowering of the freezing point) there is a fair agreement as shown in the following table:

VALUES OF i

Salt	Gram-equivalents Per Liter	Conductivity	Freezing Point	Plasmolysis
KCl.....	0.14	1.86	1.82	1.81
$Ca(NO_3)_2$....	0.18	2.46	2.47	2.47
$MgSO_4$.....	0.38	1.35	1.20	1.25
$CaCl_2$.....	0.184	2.42	2.67	2.78
$K_4Fe(CN)_6$...	0.356	3.07		3.09

3. Ionic Conductivity.—From comparison of equivalent conductivities of electrolytes it becomes apparent that there is a definite order of conductivity among the ions which compose them independent of the character of the associated ions. This is illustrated by the following table which gives the equivalent conductivity of certain salts in a dilute solution of 0.0001 N.

	Chloride	Nitrate
K.....	129.05	125.49
Na.....	108.06	104.53
Li.....	98.06	94.38

The difference between the conductivity of the chloride and nitrate is the same whatever the cation and the difference between each pair of cations is the same whatever the anion. This can happen only if K, Na, Li, Cl and NO_3 have independent values as regards conductivity.

Kohlrausch's law of the *independent migration of ions* expresses this by saying that in very dilute solutions the equivalent conconductivity is the sum of the conductivities (u and v) of the ions ($\lambda_\infty = u + v$). It is evident from a cursory examination of the table that in conductivity K > Na > Li and Cl > NO_3. *Since every monovalent ion carries the same charge this difference in conducting power must be due to a different rate of travel.*

4. Mobility of Ions.—It is apparent that from the formula $\lambda_\infty = u + v$ the conductivity of one ion of an electrolyte can be determined if that of the other is known. Also since the conductivity varies as the rate of migration, the relative mobility of the various ions is deduced therefrom. The absolute rate can be measured by direct experiment when the ion is coloured or when it produces a colour change in an indicator. The actual rate of migration is very slow. Under a fall of potential of 1 volt per cm. the

H-ion moves 0.0033 cm. per second
OH-ion moves 0.0018 cm. per second
K-ion moves 0.00067 cm. per second

Historically the argument was reversed. The fact that the charge on an ion is equivalent to its valency was deduced by Hittorf from conductivity experiments in which the ratio of the speeds of cation and anion respectively was obtained from measurement of changes in concentration round the anode and cathode. Because it is easy to prove (see textbooks of Physical Chemistry) that

$$\frac{\text{speed of cation}}{\text{speed of anion}} = \frac{\text{fall of concentration round anode}}{\text{fall of concentration round cathode}}$$

In order of speed

$$H > K > Na > Li$$

$$OH > Cl > NO_3 > CH_3COO$$

Note that size or weight of ion does not always determine the rate of migration in the way we would expect. K has an atomic weight of 39, Li of 7, yet K moves twice as fast as Li, although on the kinetic theory and from diffusion experiments in general we should expect the lighter ion to move the faster. This is apparently accounted for by the fact that ions are hydrated and the water envelope of the lighter ion happens in this case to be the greater, thus setting up a greater friction.

Mobility in relation to ordinary diffusion.—If it is the specific friction between the ion or hydrated ion and its medium that determines its mobility under a given potential gradient, we should expect an exactly similar effect on its mobility when concentration gradient is involved and osmotic pressure is the driving force. This is found to be the case. In ordinary diffusion, however, neither set of ions of an electrolyte can outstrip the oppo-

sitely charged set with which it is associated because the local excess of one over the other develops electrostatic forces which retard the speediest ions and accelerate their slower partners so that the electrolyte diffuses as a whole.

The rate of diffusion of an electrolyte is thus compounded of the speed of both ions. In any series with a common anion (chloride, let us say), the order of rate of diffusion will be the same as the order of speed of migration of the cations:

$$HCl > KCl > NaCl > LiCl$$

In any series with a common cation it will be that of the anions:

$$NaOH > NaCl > NaNO_3 > NaCH_3COO$$

5. Effects of Different Velocity of Cation and Anion Respectively—Diffusion Potential.—The speedier ion does succeed in outstripping the slower one to an excessively minute extent. At a diffusion front, therefore, if the cations and anions travel at a different speed, there will be contiguous zones in which + and − charges respectively dominate, that is, there will be a gradient of electric potential—so-called **diffusion potential.** This may happen at the boundary zone between a salt solution and (1) water, (2) a differently concentrated solution of the same salt, or (3) a solution of another salt. Anything which steepens the gradient of diffusion will also tend to steepen the potential gradient. A membrane may do so very effectively. These electrical effects will be taken up in another chapter.

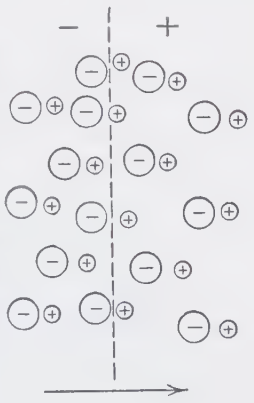

FIG. 31.

IV. SELECTIVE PERMEABILITY OF MEMBRANES

1. Relation to the Type of Membrane.—Many osmotic phenomena depend on the selective permeability of membranes. This is determined in several ways. If the membrane is a solid structure there are two possible mechanisms of permeability: (1) solu-

tion in the membrane substance, (2) passage through pores. The former is associated mainly with a homogeneous membrane, the latter with a heterogeneous one although in the limiting case when the pores are reduced to interstices between molecules the distinction ceases to exist. There will still be a difference, however, as regards the forces involved.

The term *membrane* as used with reference to cells is extended however to include a liquid layer or film. Here again we may have penetration by solution or, in the case of an emulsion, penetration through the spaces between the droplets—equivalent to the pores of a solid membrane. But a third possibility arises, namely, that the penetrating substance is adsorbed by particles suspended in the liquid barrier, or by its emulsion droplets, and that these particles through Brownian movement (see under Colloids) are carried from one side of the layer to the other and release some of the adsorbate on the other side.

(*a*) *Penetration by Solution.*—An example of a physical membrane which is semipermeable in virtue of solution differentiation is afforded by a layer of phenol lying between a (heavier) saturated solution of copper sulphate in water and a (lighter) layer of water alone, each medium being saturated as to the other. The phenol membrane is permeable to water but almost impermeable to $CuSO_4$. The lower stratum therefore gradually enlarges by osmosis at the expense of the upper. On the other hand, if some neutral red is added to the upper layer it dissolves readily in the phenol and thus is able to pass to the lower aqueous medium.

(*b*) *Penetration through Pores.*—Colloidal membranes, with which osmotic experiments are usually performed, are porous and may be prepared of all degrees of permeability. A copper ferrocyanide membrane is permeable to water and in less degree to very simple salts like NaCl, but impermeable to more complex molecules. A gelatin gel allows most dyes to pass, although it may be impermeable to the highly colloidal Congo Red. One and the same substance may exhibit varying degrees of permeability depending on its mode of preparation. Thus an air-dry collodion bag which is highly impermeable, scarcely allowing even water to pass, may have its permeability increased by causing it to swell in a suitable medium—a mixture of alcohol and water. The greater the proportion of alcohol the greater the swelling and the greater the resulting permeability. Increased permeability is

indicated not only by a more rapid diffusion of water, etc., but by an increase in the size of the molecules which are allowed to pass. This can be interpreted only as pointing to an increase of pore size as the membrane enlarges. At one end of the series membranes swollen in a low concentration of alcohol are slowly permeable to water and in a lesser degree to simpler electrolytes such as NaCl, KNO_3, but to nothing more complex. At the other end membranes swollen in a high proportion of alcohol allow even highly colloidal substances like starch and aniline blue to pass. The permeability of a porous membrane to ions depends also on its *electric charge*, as will be discussed later.

(*c*) *Penetration by adsorption.*—The slow diffusion of colloids renders difficult a macroscopic demonstration of transportation of substances adsorbed by them, although in films of ultramicroscopic thickness which we find in the living cell the kinetic activity of particles becomes relatively important. We may, however, present an analogy by using fall under gravity instead of diffusion as the mode of transport of the particles. As an experiment, carbon may be shaken up with a methylene blue solution till the medium appears colourless. The filtered paste may be emulsified with oil to make it float on water. We then set up in a test tube three successive strata, phenol, water and the above emulsion. Carbon particles gradually detach themselves from the top layer and fall to the bottom layer, which soon becomes blue by dissolving dye from the carbon surface.

2. Relation to the Propelling Force.—In estimating permeability one must take count of the driving force. When this is osmotic pressure (diffusion pressure) the force is proportional to the difference of concentration on the two sides of a membrane. If the rate of passage is always proportional to the driving force the membrane may be described as having a definite permeability irrespective of the concentration of solute. But there are cases where this is not so; for example, in passage by adsorption, because the amount adsorbed is not proportional to the concentration in solution. Also in the passage of ions when the driving force is electrical potential the rate is not proportional to the difference of potential between the two media because the banking up of ions on the respective sides of the membrane tends to set up a potential gradient in the opposite direction which retards the flow. The permeability of a porous membrane to ions must also

be quite different when the driving force is electrical potential as compared with when it is osmotic pressure. In all such cases we cannot speak of a membrane as having definite permeability since this varies with the nature and magnitude of the driving force. The significance of these facts with reference to cell permeability will be evident later.

CHAPTER VIII

DIFFUSION AND OSMOSIS IN CELLS

The subjects to be discussed under this head are wider than the above title indicates. The exchange of substances between cells and their medium is more than a simple diffusion process and the pressures set up by cells are not entirely osmotic. Yet these are undoubtedly dominant factors, and a good way of studying the physiological phenomena referred to is to see how far they can be explained by these factors and how far not.

I. CELL PERMEABILITY

1. Definition of Cell Permeability.—Cell permeability as an important factor in many physiological processes is continually under discussion but the term is used vaguely with varied implications. Some definition is necessary though there is none to which everyone will subscribe. We have seen that even with physical membranes and without any change in the membrane itself permeability is not a constant property but varies with the nature and magnitude of the force which causes the substance to pass through. Furthermore, in protoplasm autonomous forces, which we cannot estimate, may be at work, over and above those which may be imposed from without. When the protoplasm takes an active part in the process we speak of the condition as " active or physiological permeability " as opposed to " passive or physical permeability " where the protoplasm functions merely as an inert membrane. It is somewhat stretching the term permeability to apply it to active transport of substance but our inability to distinguish active from passive passage seems to necessitate the inclusion of both as Cell Permeability.

It is incorrect, however, to speak of permeability as synonymous with the mechanisms of absorption and secretion by cells. Absorption may depend on *storage* and secretion on *release* of sub-

stance at the end or respectively beginning of its journey, as well as on the movement in or out of the cell which alone belongs to permeability. In vital staining, for instance, the dye is often removed from solution by adsorption or chemical combination in vacuoles or granules. This greatly increases the amount which can be taken up and it also increases indirectly the rate of absorption by maintaining the concentration gradient. But what happens at the end or beginning of the path has nothing to do with permeability as such.

In keeping with what has been said we may for the moment define *the* **permeability of a cell to any substance** *as the property of its protoplasm (or protoplasm plus cell wall) which is measured by the rate at which that substance passes through it under a given environmental driving force.* No assumption is made as to the mechanism of passage. Ordinarily the external force with which we are concerned is osmotic, depending on the difference of concentration at the starting and end points respectively—as in the external medium and cell sap of a plant cell; but permeability to ions may also be estimated under a given potential difference. Ordinarily the cell wall has little to do with the selective permeability of cells, being permeable to any kind of molecule below colloidal size. Cutinized and suberized walls, however, are relatively impermeable even to water and gases. The discussion which follows refers therefore to protoplasm only.

The next question is how cell permeability as above defined may be measured.

2. Measurement of Permeability. (*a*) *Osmotic methods.*—Plasmolysis indicates that a cell is more permeable to water than to the solute molecules; but if the latter penetrate at all they gradually add to the solute inside, causing the cell to reabsorb water. The application of this behaviour to measurement of permeability is based on the assumption that the rate of penetration of solute varies in proportion to the rate at which the total amount of solutes inside the cell increases. There are two main variations of the method. The **deplasmolysis** test is to plasmolyse the cell with the solution whose penetrability is to be measured—or, if this penetrates too rapidly for plasmolysis, with a mixture of it and cane sugar—and then to observe the rate of recovery from plasmolysis, termed deplasmolysis. The osmotic pressure inside at equilibrium remains equal to the osmotic pressure outside;

therefore the volume of the cell sap increases in proportion to the increase in amount of osmotically active solute contained in it.

The **osmotic value** test is to observe the same type of cell in a series of solutions of graded concentration, noting as time proceeds in which concentration the cells are just beginning to plasmolyze. As the amount of solute in the cell increases, successively higher concentrations outside are isotonic with the sap at incipient plasmolysis.

As a measure of permeability the method involves various assumptions which have been strongly criticised by Stiles, Iljin,[1] and others, especially (1) the assumption that the change in osmotic value of the cell sap is directly due to the osmotic pressure of the solute which penetrates and (2) that the solute which penetrates remains osmotically active. It has been shown for example (Iljin) that potassium ions cause hydrolysis of starch to sugar in guard cells. Small changes in H-ion concentration may have even more profound effects. Stiles lays stress on exosmosis and adsorption as disturbing factors. Such possible sources of error have not been ignored by experimenters and it seems to be the case that at least with some kinds of cell and substance these errors are not always important. Non-electrolytes penetrate *Tradescantia* cells, according to Fick's law of diffusion, until the concentration inside equals the concentration outside, and they escape at about the same rate as they enter (Barlund[2]).

Another objection to osmotic methods is that even if they measure permeability, yet under the conditions, permeability is abnormal. Sucrose which often enters slowly in plasmolysis experiments does not diffuse appreciably out of the normal cells when bathed by water. It would seem that a hypertonic solution even of sucrose and still more of certain salts must slightly increase permeability when applied to the exterior of the cell, not enough to invalidate the deplasmolysis method with reference to substances which penetrate more or less easily, but enough to render questionable the application to the normal state of cells of the results obtained for less penetrable substances. In other words, these experiments do not prove that it must be something other than semipermeability which prevents sugars and salts from leaching out of healthy cells into tap water.

[1] Biochem. Zeitsch. 132: 494, (1922).

[2] Acta Botanica Fennica 5 : 1, (1929).

(*b*) *Methods depending* on a *visible change* in the cell interior, for example, in penetration of dye, of acid or alkali when an indicator is present, or of substances reacting to produce an intracellular precipitate. As a qualitative test such methods are simple and demonstrative, but as a quantitative measure they can be used only when there is no accumulation by adsorption or chemical binding. Plant cells afford the best subject because penetration into the vacuole can be observed, but even vacuolar sap frequently contains adsorbent colloidal matter and organic acids which react with basic dyes, etc.

(*c*) *Chemical methods* employing *analysis of medium* or of *medium and sap*.—Change in concentration of a solute in the external medium alone is unreliable because this may be due partly or entirely to adsorption on cell walls and surfaces. The most valuable results are obtained with material such as *Nitella* and *Valonia* with very large cells from which sap uncontaminated by protoplasm can be extracted and its composition compared with that of the medium.

(*d*) *Conductivity Methods*.—Used simply as a measure of changes in electrolyte concentration in the medium the conductivity method fails to detect exchange of ions which is one of the most important modes of penetration. It is when adapted to measure the resistance, or rather impedance, of the cells themselves that electrical methods have given the most unique and valuable information regarding permeability. Interpretation of the results is very difficult however and involves certain debatable assumptions. Discussion of these is taken up later.

(*e*) *Physiological Methods*.—Sometimes penetration of a substance is revealed by a physiological response. The method is singular in two ways: (1) It may be far more sensitive than any physical test, and (2) it applies to penetration into protoplasm and not into the vacuole. It can only be used qualitatively or comparatively and is rarely available. The response to various cations by *Spirogyra* cells is an example.

In short, almost every kind of determination of presumed permeability is liable to be influenced by other factors than that which it is desired to measure and it is only by checking one method against another to find points on which they agree that we can formulate any reliable conclusions.

3. The plasma membrane hypothesis.—The observation of permeability phenomena led long ago to the now classical doctrine of a plasma membrane, which, however, is not universally accepted. The theory that the selective permeability of protoplasm is a function not of the whole cell but of a superficial layer termed for convenience the plasma membrane may be regarded as demonstrated for certain types of cell and undecided for others.

The term " membrane " is not meant to imply a solid structure but merely a surface layer differentiated in some way from the interior. This layer may perhaps merge gradually into the inner cytoplasm or may be simply a monomolecular film. The plasma membrane is not the secreted envelope by which plant cells and many animal cells are covered; nor is it necessarily coextensive with the more consistent " ectoplasm " which is tangibly demonstrable in some cells. It is a film which is assumed to possess " semipermeable " property, and it is the localization of this property that requires proof. A few points of evidence are as follows:

From the analysis on p. 91, we see that there is no Na in the interior of red blood cells, although there is a good deal dissolved in the surrounding blood plasma. The reason is not that Na ions cannot dissolve in the interior of the cells as is shown by the fact that K and Cl ions are in solution there. The prevention of the entry of Na must in some way, therefore, be a function of the superficial layer.

Again it is found that dyes such as eosin which do not enter many cells under normal conditions, nevertheless when injected beneath the surface, diffuse freely through their interior to the external boundary but do not pass out as shown by Chambers for sea urchin eggs and *Amoeba*. The method has not been applied to plant cells but the fact that disturbance of the surface by plasmolysis or deplasmolysis sometimes allows acidic dyes to enter easily during life favours the membrane hypothesis.

Also, as first shown by de Vries, the lining of the sap vacuole of plant cells may act as a membrane which remains virtually impermeable to plasmolyzing agents and to dyes after the rest of the cell is dead. Under normal conditions also this same structure must protect the protoplasm against toxic substances dissolved in the sap. For example, the concentration of acid in the vacuole of many plant cells and probably sometimes in the diges-

tive vacuoles of protozoa, is such as to kill the cells rapidly if applied externally.

Another type of evidence is based on the behaviour of protoplasm when a fresh surface is exposed. As was noted in connection with concentration membranes, the internal protoplasm may mix with water unless a fresh film forms over it. The ability to extend or repair the plasma membrane varies greatly with the type of cell. At one extreme is the condition described by Lepeschkin for *Bryopsis* (a coenocyte) in which the living substance may be almost indefinitely emulsified without losing its essential quality. In such cases the bulk of the protoplasm must be, potentially at least, a plasma membrane. The other extreme is shown by red blood cells in which very moderate extension of the original surface film causes loss of semipermeability—for instance, in hemolysis by hypertonic solutions.

It is plain that only as much of the protoplasm as is, when in liquid form, immiscible with water can exhibit the selective permeability that the cell does. A hydrosol or a protein gel differs little from water as a diffusion medium and the indications are that a large part of most cells is hydrosol in character. On this ground also it seems necessary to refer selective permeability to a plasma membrane rather than to protoplasm in general.

4. Thickness of the plasma membrane.—The microinjection experiments referred to above show that the semipermeable layer is invisible but the only evidence as to its actual dimensions is derived from conductivity measurement. Since ions are the carriers of electricity in a solution, the conductivity of protoplasm is in some measure an index of its permeability to ions, although it tells us little as to the nature of the ions that penetrate or whether any ions do not penetrate at all. It is found that the conductivity of entire cells is low relative to that of the internal protoplasm or cell sap, each of which frequently ranges about that of a decinormal KCl solution, as measured for example in hemolyzed blood cells or in extracted cell sap from plant cells. The high resistance of the cell as a whole must therefore be localized in the superficial layer. The impediment which this layer offers to the passage of electricity is reduced when an alternating current of high frequency is used until at very high frequencies—1,000,000 cycles and over—it practically disappears. The range

of oscillation of the ions then becomes so small that few of them hit the barrier.

With lower frequencies the barrier acts not only as a *resistance* but also as a condenser—that is, it exhibits *capacity*—because ions become piled up on both sides of it and set up an opposing electromotive force (see later under Polarized membranes). By matching the **impedance** (resistance + capacity) of cells against a suitable combination of resistance and capacity in series, so that the respective impedances are equal at all frequencies, the capacity as well as the resistance of the cells may be estimated. Under certain conditions the capacity per unit area of cell surface may also be be determined. From this the thickness of the condensing layer could be calculated if the dielectric constant of its substance were known. This, of course, is not known, but it must lie somewhere between fatty oils (3) and water (80)—probably near the former—so that the *possible* range of thickness can be estimated. Taking a provisional figure of 10, the thickness of the plasma membrane of blood cells is estimated by MacClendon[1] to be of monomolecular dimensions. With somewhat greater possibility of error the condensing layer or layers on the individual cells of beet tissue is estimated by Remington[2] to be about 10 millimicrons thick. This thickness must be divided between the external and vacuolar membranes. It is of ultramicroscopic dimensions and far less than the total thickness of the cytoplasm, yet much greater than in the case of blood cells. It is significant in this connection that blood cells should be so easily hemolyzed by swelling in hypotonic solutions. Conductivity experiments thus agree with those of microinjection in pointing to a plasma membrane of invisible thickness and further indicate that the thickness varies in different kinds of cell.

5. Semipermeability of the Plasma Membrane.—The relation of volume and pressure in most plant cells to external osmotic pressure shows that they act as osmotic machines and therefore must possess a degree of semipermeability. The general agreement in results of de Vries's plasmolytic method with those of physical methods of comparing osmotic pressure of many types of solution is a case in point. The procedure assumes that electro-

[1] Protoplasma 3:71, 1927.
[2] Protoplasma 5:338, 1928.

lytes and non-electrolytes which cause a cell to take up the same volume have the same osmotic pressure. This is true only if the cell is semipermeable to the solutions. The influence which they might have on cell volume if they penetrated, namely, that on the swelling and osmotic pressure of the cell colloids, would follow an entirely different order, H-ion concentration and the lyotropic property of the ions being the predominant factors (see under Colloids, p. 166).

Animal cells also change volume in response to changes of osmotic pressure. In 0.7 per cent NaCl and other isotonic solutions, frog's muscle undergoes no change in weight even after several hours, showing that muscle is practically impermeable to the salt. We find evidence, however, from plasmolysis results that permeability is modifiable as well as selective. Although toward certain plasmolytes, such as cane sugar and $CaCl_2$, cells often maintain almost complete impermeability, toward others to which in nature they may appear semipermeable this is not usually the case. But the conditions in plasmolysis are abnormal and such as tend to increased permeability for other things besides the plasmolyzing agents themselves. Therefore the possibility of more complete semipermeability for the same agents under other conditions is not disproved. The results do demonstrate that the permeability or respectively semipermeability of protoplasm is not fixed but varies with the type of cell and with the environment.

The evidence from osmotic relations as to the semipermeability of protoplasm or its plasma membrane is supported by analyses of the concentration and distribution of common substances inside and outside of cells. Protoplasm as a rule is freely permeable to water but maintains indefinitely an unequal concentration of sugars, acids, salts, etc., on either side of it (in plant cells) and also within as opposed to outside itself. For example, the sugar and pigment in beet and red onion cells do not diffuse out when these are placed in water. Even in the case of cells which are all their life bathed by water a highly unequal ratio is maintained. Thus in the vacuole of *Nitella* (the large cells of which permit extraction and analysis of sap apart from protoplasm) nearly all the inorganic ions are in vastly higher concentration than in the water in which the plant lives. Of anions Cl is about 100 times, and SO_4 nearly a thousand times, whereas of cations K is several

thousand times more concentrated inside than outside the cells. In the case of cells living in a more saline medium the distribution of concentration of some ions may be reversed, that is, greater outside. Thus the ratio of SO_4 in sea water to that in the sap of *Valonia* (a giant "celled" coenocyte) approaches 1000 to 1. Mg, Ca and Na are also higher in concentration externally, but on the other hand K is as usual much higher internally. In both these cases there is little protein in the sap and the ions are osmotically and electrolytically free.

Turning to animal cells, first to the example of red blood corpuscles, the distribution of K and Na in the case of the rabbit, according to Abderhalden is:

	Plasma	Corpuscles
Potassium	0.259	5.229 per 1000
Sodium	4.442	0

It has been shown that the K is mainly free from combination and the Na almost entirely so. Similarly the Na of the blood is unable to diffuse into muscle cells and other parts of the body while K is stored up in the tissue.

We must conclude from facts such as those cited one of three alternatives: (1) that protoplasm is normally impermeable in a physical sense to the solutes in question or (2) that it is continually exerting energy against the force of diffusion, or (3) that it varies between these two conditions from time to time. In the case of inert cells like mammalian red blood cells there is no evidence that any but the first alternative holds. In more active cells there is evidence that the third alternative may apply. Thus it is found that *Nitella* may accumulate Cl ions or exchange Cl′ for Br′, but the action depends largely on light. It would seem a wasteful and inefficient arrangement for cells to do work continuously when the simple mechanism of semipermeability could avoid it.

Permeability varies in degree and character in different types of cell. Thus although the penetration of simple anions in *Nitella* is extremely slow and conditional it is rapid and free in blood cells and perhaps other cells of the animal body. Or again although with many cells the penetration of acidic dyes can scarcely be detected or not at all, it is rapid in the case of internal tissues of some plants and a few animal tissues. Perhaps it is the rule that cells exposed to a variable chemical environment possess greater

selective permeability than those, such as cells in the interior of plants and animals, which live in an almost constant medium.

We may say that protoplasm at least acts as if it were semipermeable; but then we must add that it does not always do so. Most of the solutes in a cell have accumulated during its lifetime, therefore at some period and in some way they must pass through protoplasm and work must have been done on those which are concentrated in the cell. Again, if the chemical environment is changed a redistribution of ions often takes place. The same ions toward which protoplasm appears semipermeable and others which it does not commonly meet with may be found to enter or leave the cell. Protoplasmic permeability toward substances to which it is sometimes in effect semipermeable must be regarded as readily modifiable.

Penetration of such substances is not proportional to the concentration gradient and sometimes proceeds in opposition to it. All of which has naturally led to the term **physiological** for this kind of permeability. This does not mean that entry or exit of such substances is always regulated by the needs of the cell—because it is not—but that the mechanism is highly complex and sometimes involves expenditure of energy.

The next question is how far the mechanism of permeability can be elucidated on known physical principles.

6. The physical mechanism of permeability.—Although our ideas of the mechanism of permeability are only hypothetical, a consideration of the fundamental facts and a reference to the more outstanding theories based upon them will be useful. These theories are founded on inference from: (*a*) The composition and behaviour of protoplasm; (*b*) The factors that modify permeability; (*c*) The relation between the chemical character and penetrability of substances in general.

(*a*) *Inference from the Composition and Behaviour of Protoplasm.*—Protoplasm is essentially a colloidal complex of water, protein and lipoids—with or without carbohydrates. In the interior of the cell, water is usually the external phase and the organic particles are in large measure free to disperse in water; at the cell surface a water-immiscible phase is either external or so closely packed as to be coherent. The surface layer is commonly liquid or at least plastic, but must itself be colloidal since it is so markedly affected by electrolytes, especially di- and trivalent cations,

and can so readily gelatinize and again liquefy. Also it must dissolve or imbibe water since the latter can pass through. The extreme surface, according to physical analogy, may be expected to be the seat of a certain concentration and probably orientation of the more surface active elements but it is not safe to assume that activity at the protoplasmic surface runs parallel to that at a water-air surface.

(*b*) *Modification of Permeability.* Some further insight into the mechanism of permeability is obtained from consideration of the factors which alter it. In the first place, no kind of influence profoundly affects the penetration of substances which enter most easily. This is in harmony with the theory that these penetrate by solution in the membrane substance.

As regards the penetration of compounds, to which protoplasm is not always permeable, we may consider first the effect of inorganic salts. Of these it is the cations alone that have a marked effect. Bivalent and trivalent cations, unless toxic, diminish permeability. In absence of any polyvalent cations in the external medium, *Spirogyra* cells die within an hour or so, while the plasma membrane of naked cells is sometimes seen to disintegrate. In nature the stabilizing rôle is filled by Ca, but Sr, Zn, and other of the less toxic cations can temporarily replace it. Salts of alkali metals, especially Na and Li, are commonly found to increase permeability and lead to disorganization of the plasma membrane.

The mutual antagonism of mono- and polyvalent cations (like Na and Ca) is generally regarded as due to opposite effects on permeability; the antagonism of Ca or other non-toxic cations to the action of low concentrations of more toxic ions of whatever valency, is presumably the result of the former being supplied in sufficient concentration to lower permeability and keep the others out. Equally they tend in such concentrations to keep themselves out. To understand how ions affect permeability it is necessary to anticipate a little of what will be noted in dealing with colloids.

The liquefying and disintegrating effect of Li, Na and K ions on the plasma membrane is duplicated by their action on certain physical colloids. In both cases the particles become separated—possibly by taking up more water through adsorption—until finally they disperse into the surrounding medium. Bivalent and trivalent cations on the other hand tend to bring the particles closer together, closing up the interstices. Higher concentrations

of these ions, especially the more adsorbable ones, cause the micellae (particles) to group into solid clumps with gaps between (coagulation) so that the semipermeability of the membrane is destroyed in another way. This change is not reversible. The injury is irretrievable.

It appears from all this that the various effects of ions on permeability agree very well with the conception of the plasma membrane as a colloidal ultrafilter.

Another notable chemical agency influencing permeability is that of narcotics. In those concentrations at which their narcotic action appears, they lower permeability. In toxic concentrations they irreversibly raise it. Their activity as we have seen is practically proportional to their lowering of surface tension and they are probably adsorbed by the plasma membrane. One theory of their action is that by displacing the adsorbed water from the surface of the micellae they reduce the size of the pores to polar substances. In higher concentrations they coagulate the membrane as they do physical colloids of a similar nature.

Although ploy valent cations and narcotics are the principal agents that lower permeability below normal, there are many factors besides salts of monovalent cations that raise it, for example heat, light, electric currents, mechanical action, solutions of high osmotic pressure, and in special cases various chemical substances.

(*c*) *Relation between chemical character and penetrability of substances.*—The most important generalization in this connection is the rule that the more polar the molecules of a substance the poorer its penetrating power. To mention a few examples, hydrocarbons, the least polar of all compounds, penetrate instantly. Nor does increasing length of the hydrocarbon chain or diameter of the ring have any retarding effect. If a single polar group is united with a hydrocarbon radical, penetration is still rapid though slower than the above. As the number of polar groups is increased the rate is correspondingly reduced. (See Jacobs in "General Cytology.") Take for example the various alcohol series:

Ethyl Alcohol $C_2H_5(OH)$ (one polar group)—penetration too rapid for plasmolysis.

Ethylene Alcohol $C_2H_4(OH)_2$ (two polar groups)—temporary plasmolysis for ten to fifteen minutes.

Glycerol $C_3H_5(OH)_3$ (three polar groups)—temporary plasmolysis lasting for several hours.

Increasing size of molecules in the alcohols as in the hydrocarbons is not a retarding factor, but rather in so far as an increase in the length of the hydrocarbon chain causes nonpolar properties to predominate it facilitates their entry.

Carbohydrates appear to have much the same penetrating power as alcohols which have the same number of C atoms and of attached polar groups.

Just as the introduction of polar groups OH, COOH, NH_2, etc., into hydrocarbon chains decreases penetrating power, conversely substitution of H in these polar groups by a non-polar radical like CH_3 (methyl), C_2H_5 (ethyl), etc., increases it. Urea, $CO(NH_2)_2$, penetrates slowly enough to give plasmolysis of considerable duration; ethyl, diethyl and methyl urea penetrate in rapidly increasing ratio.

The above rule as to preponderance of hydrocarbon chain and polar groups, respectively, appears to hold good for organic acids and bases also, but a modification of it, namely, that solutions of electrolyte penetrate in proportion as they contain non-dissociated molecules, also harmonizes with the broad facts. The weak fatty acids, acetic, etc., penetrate rapidly, the strong mineral acids, very slowly, and often the result can only be detected after they have injured the cell. A similar distinction holds when weak alkalis like ammonia and the alkaloids (caffeine, etc.) are compared with strong alkalis.

The cases of carbonic acid, H_2S and ammonia, are peculiar in that the free anhydrides (CO_2, H_2S and NH_4) exist in the respective solutions and these like most other gases (O_2, N, etc.) are very freely permeable. These reagents may cause the intra- and extracellular H-ion concentration to vary quite independently. NH_4 for example may diffuse out of a cell (as of *Elodea*) into a dilute NaOH solution and cause the cell sap to become acid as it normally is. CO_2 also may enter from an alkaline medium and produce a more acid reaction inside the cell.

Penetration of Polar Compounds.—Amid the many complicating factors affecting permeability of polar compounds it appears that one in particular, which is of little account in the case of predominantly apolar molecules, is of considerable importance in these, namely, their size. Thus water, among the simplest of the polar molecules, at least of those presented to the cell in a non-dissociated form, usually finds a free passage, although cases are

known in which cells are for a time impermeable to water even. The molecules of many inorganic salts are also fairly simple, but the high percentage of dissociation in aqueous solution militates against their entry—since, apparently, ions pass less readily through the membrane than undissociated molecules. This too may depend partly on size, since ions are more hydrated than molecules. Also, considering the ions themselves, monovalent penetrate better than polyvalent, whereas in the same group the less hydrated and therefore smaller units penetrate better than the more hydrated. Thus the order of penetration among the alkali metals is usually Cs > Rb > K > Na > Li. In the case of ions the *sign of their charge* and general *adsorbability* seem also to play a part—a point to be dealt with more fully later.

When we come to the large organic molecules, penetration is usually difficult to demonstrate but there is again distinct superiority in permeability of smaller molecules: (*a*) among dyes, especially acid dyes; (*b*) of pentoses and hexoses as compared with sucrose among the sugars; and (*c*) of the smaller as compared with the larger amino-acid molecules; while colloids such as starch and the proteins, even though in molecular solution, are quite incapable of entry, as the fact of their preliminary digestion implies.

Differences in the Penetration Mechanism of Polar and Apolar Compounds.—A summary comparison of the factors that determine the penetration of apolar and polar compounds respectively will make it evident that there is a profound difference in mechanism in the two cases:

Apolar	Polar
Rate of penetration	*Rate of penetration*
1. Does not diminish with molecular size.	1. Diminishes with molecular size.
2. Is usually more or less proportional to external concentration.	2. Does not increase proportionally to the concentration (due partly at least to the effect of the agent itself on permeability).
3. Increases very slightly with temperature.	3. Increases greatly with temperature.
4. Is not much affected by other factors (light, heat, chemical agents, etc.).	4. May be modified greatly by such agents.

What is the significance of these facts as to the mechanism of permeability? The symbasis between apolarity and penetra-

bility may point either to solution or adsorption by the plasma membrane. The rule in solution is "like to like." Polar substances dissolve in polar liquids such as water—apolar in apolar organic liquids such as benzol, xylol, ether chloroform. It is possible therefore to regard the plasma membrane as an apolar solvent. But general capillary activity or adsorbability in aqueous solution also goes along with apolarity. Two classical theories of permeability adopt these alternative views. We shall consider them first.

7. Theories of Cell Permeability. (*a*) *Overton's Lipoid Solution Theory.*—The original classification of substances according to their penetrability was made by Overton who tested some 500 or more and on the basis of his classification put forward the lipoid theory of permeability. His thesis was that lipoid-soluble substances penetrate protoplasm, lipoid-non-soluble ordinarily do not (apolar and polar are later terms). As we have seen, Overton exaggerated the inability of the latter class to penetrate. "Lipoids" were specified since this class of substances is the only supposedly invariable constituent of protoplasm that possesses the required solution affinities. Of these, phospholipins (lecithin and cephalin) and sterols (cholesterol, phytosterol, etc.) are regarded as the most important. The amount of these substances in plant protoplasm, however, is very small, if indeed they are present at all. Nevertheless if localized in the plasma membrane a very small amount would suffice. Later modifications of Overton's theory regard the barrier not as pure lipoid but as a complex of lipoid and protein, etc., with apolar solution affinities. Attempts to imitate the plasma membrane by artificial systems might not be expected to be very successful since the organization can hardly be duplicated, even if the composition could. Nevertheless, MacDougal and Moravek[1] have constructed a colloidal cell in which the combination of cholesterol and lecithin with gelatin formed a membrane substance, impermeable to H- and OH-ions, while allowing sugar, K-ions, Cl-ions and water to pass.

Overton based his theory in considerable measure on the penetration of dyes, finding as he thought that penetration or non-penetration depended on lipoid solubility. Another factor is the degree of dissociation. Rate of penetration increases with the number of undissociated molecules in the solution. Thus for

[1] D. T. MacDougal and V. Moravek, Protoplasma 2, 161. (1927.)

example a more alkaline reaction of the medium in the case of basic dyes, a more acid one in the case of acidic, and a reaction approaching the isoelectric point of amphoteric dyes such as methyl red, all facilitate entry, probably because they reduce dissociation of the dye. Similarly it has been discovered in practical experience that acidic dyes with a low dissociation constant may penetrate cells which are impermeable to those with a higher one. There is evidence that inorganic molecules also penetrate better than ions. Since these substances dissolve as molecules in organic liquids the facts are not unfavourable to the lipoid solubility theory. But they do not disallow other possibilities.

(*b*) *Traube's Adsorption Theory.*—An important objection to Overton's theory is the ready penetrability of basic dyes such as methylene blue which are poorly soluble in lipoid solvents and which do not dissolve in hydrated lipoids, but are only *adsorbed* by them. It was also shown by Traube that narcotics which penetrate readily obey the adsorption law in the relation between concentration in the cell and in the medium. If in the cell as a whole narcotics are adsorbed more than dissolved, why not in the plasma membrane also? In speculating on how adsorption can result in penetration we may recall the opposite result in the diffusion of dyes through filter paper and in many other filtration experiments. The adsorbed substance clings to the filter and often tends to block its pores so that the passage not only of itself but of other substances is inhibited. A gross solid membrane however is scarcely a good analogue to an extremely thin and liquid one, in which the molecules or micellae may be in a state of kinetic activity. Also with certain types of physical membrane permeability is related to adsorption in the way assumed for the plasma membrane. Positive adsorption facilitates penetration, negative adsorption prevents it. It is evident therefore that adsorption does not necessarily imply immobility of the adsorbate even in a solid membrane. Since in this case particle size is also a factor, further discussion is deferred to a later paragraph.

In our ignorance of the precise nature of the plasma membrane it is impossible to decide between the lipoid solubility and adsorption theories as applied to apolar compounds. On the whole however there is rather better correlation with lipoid solubility than capillary activity (at an air surface at least). The fact that

the penetration of apolar substances is so little affected by agents that modify the colloidal state of the membrane also favours solution rather than adsorption. After all, if the membrane is colloidal the distinction is hard to draw between solution in the particles and adsorption at their surface, and it is of theoretical rather than practical importance.

The case of the more polar substances presents features that have given rise to other theories.

(*c*) *The Sieve or Filter Theory.*—As first shown by Küster, tissue cells of higher plants are often freely permeable to acidic dyes. Ruhland showed that the rate of penetration of these and also of many basic dyes varies inversely as the size of the diffusing particle. It does not appear that the permeability of the cell walls alone accounts entirely for this result. As we have seen, a correlation between particle size and penetration may be extended to polar substances leading to the theory that the mechanism of permeability as far as these as concerned is that of passage through the pores of a plasma membrane which acts as a micro-sieve or ultrafilter.

(*d*) *Capillary Theories.*—It is evident, however, that the sieve theory is incomplete unless it takes count of adsorption, both mechanical and electrical. We accordingly find it modified as *capillary and electrocapillary theories* of permeability, especially as applied to ions.

Experiments of Michaelis with collodion membranes of suitable pore size and with apple skin show that negatively charged membranes may allow cations to pass while holding back anions of no greater size. Presumably ions of the same sign as the membrane are unable to approach within a certain small distance of the walls of the pores. Thus the effective diameter of the pores is reduced for these ions and may be too small for them to enter, whereas ions of the opposite sign which can use the whole width of the space may still pass through even though larger than the former ions.

The plasma membrane of blood cells and possibly of other cells in the animal body is impermeable to inorganic cations but permeable to the smaller anions. For application of the capillary theory in this case it is not necessary that the plasma membrane of these cells should carry a positive charge, since at an indifferent surface anions are more capillary active than cations. With other types of cell, however, the evidence indicates a relatively more rapid absorp-

tion of certain cations than of anions (see under Bioelectric potential). Trivalent cations even in extremely dilute solution rapidly produce marked (reversible) changes in the interior of *Spirogyra* cells. Noble metals and many divalent ions are also active. If the speed with which the reaction is produced by one of these ions is plotted against its concentration in the solution outside the cell, the curve bears a resemblance to the curve of adsorption *v.* concentration.

In short, no one theory fits all the facts of permeability, but if one considers the plasma membrane as a fine colloidal or molecular ultrafilter it will be seen that all the above mechanisms may operate in combination. A diagram will best illustrate this. (Fig. 32.) Let the membrane be represented as consisting of packed micellae or large molecules (*m*) (arbitrarily represented as spherical) with films of bound water (*b. w.*) and a certain amount of free water (*f. w.*) in the interstices. If these micellae are stationary large molecules will only penetrate the membrane in proportion as they dissolve in the micellae themselves, in other words, in proportion as they are non-polar. Substances which do not dissolve in the micellae (polar compounds) may penetrate if their molecules or ions are small enough to go between. If positively adsorbed, the whole diameter of the pore is available; if negatively adsorbed, only the free water space. If, however, the micellae are in a state of kinetic activity, substances, non-polar or polar, which adhere to them may be carried across and released on the opposite side.

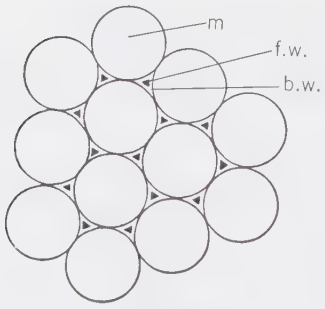

Fig. 32.

The action of agents which modify permeability (p. 93) is also intelligible according to this picture. Polyvalent cations tend to dehydration and closer packing of micellae, therefore to reduced permeability for polar compounds. Narcotics may have the same effect or may displace the adsorbed water films. Salts of the alkali metals in suitable concentration have the opposite effect. Antagonism of Na and K to polyvalent cations in permeability phenomena is thus explained. Or with respect to adsorbed substances per-

meability may be modified by arrest or acceleration of the kinetic activity of the micellae. Mass movement of the cytoplasm (cyclosis) is another possible factor. There is evidence that it functions also in translocation of substances from one end of a cell to the other. Another property of the plasma membrane which must influence its permeability to ions is its electrical bipolarity which is discussed later.

Some favour a somewhat different picture of the mechanism of permeability change, that of reversal of an emulsion.[1] The particles of our diagram are supposed to be droplets, which may fuse and become the external phase, as in Clowes's experiments with oil and water (p. 156). On this view, however, water, since it can always pass through, must dissolve in the substance of the organic phase, so that the hypothesis of a simple oil-water emulsion at least cannot stand.

8. Significance of Permeability.—The only physical explanation available of cell turgor—and all that depends on it, such as rigidity, growth and movement—is semipermeability. Retention of soluble foodstuffs and the necessary salts within the cell also finds no other explanation that we can understand. Although selective absorption of solutes by cells is no doubt largely determined by chemical relationships and to some extent by the needs of the cell, permeability is also demonstrably a factor. Alcohol is rapidly absorbed, sugar is not; yet the former is a poison, the latter a food. Cl ions penetrate cells more rapidly than SO_4 ions; yet the former do not enter into the composition of protoplasm and are not required, whereas SO_4 ions are essential. The distinction in both cases is due to difference of permeability in a purely physical sense. Chemical equilibrium with the environment is undoubtedly a feature of the life of the cell in many of its aspects, but equally characteristic according to the view maintained in this chapter is the partial shutting off of protoplasm from its chemical environment, and of one part of the cell from another, by membranes of selective permeability.

II. OSMOTIC RELATIONS OF CELLS

1. Osmotic Balance with the Environment.—Solution of inorganic salts, sugars, etc., when applied to cells, tend to make them

[1] See, for example, H. Dixon and T. A. Bennet-Clark. Nature *124*, p. 650, (1929).

lose or take up water according to the concentration used. In the case of a naked cell the volume changes; in the case of a walled cell the rigidity of the envelope usually prevents much change of volume—unless of course in the state of plasmolysis when the contracted protoplast is virtually a naked cell. The effect of a rigid wall is to produce variations of pressure instead of volume as the concentration of the external medium is changed. It is found that different kinds of solutions of the same osmotic pressure cause a naked cell to take up the same volume. This proves that these solutions are acting osmotically and that the cell is semipermeable to them. Prolonged exposure may reveal that the semipermeability is not absolute but that does not affect the argument as to the mode of action during a short experiment.

The volume which a cell tends to assume is such that the internal shall be equal to the external pressure. The external pressure which we are considering is osmotic. The internal may be partly osmotic and partly swelling pressure (imbibition). Swelling pressure is a property of gels and may therefore be displayed by the cell wall, by the protoplasm, and in certain plants rich in mucilage, even by the vacuole. On the other hand, in so far as protoplasm and sap are solutions, whether crystalloidal or colloidal, they are usually said to exert osmotic pressure. Fundamentally the pressure of a concentrated solution is largely of the same nature as swelling pressure, due mainly to attraction of the solute molecules for water rather than to their kinetic activity. The distinction between the osmotic pressure of concentrated solutions and the swelling pressure of gels is therefore somewhat arbitrary and for many purposes unimportant. Also it is to be noted that the pressure in all parts of a cell is necessarily the same whatever its origin. Pressure in the cytoplasm though it may be mainly swelling, is equal to that in the vacuole though mainly osmotic, and at equilibrium each is equal to the external pressure. The combined swelling and osmotic pressure of a cell is thus frequently referred to simply as osmotic pressure or as having a certain osmotic value, equal, namely, to the molar concentration of cane sugar which is isotonic with it.

2. Measurement of Osmotic Value.—In the case of a naked cell a non-penetrating solution which causes no change in volume is isotonic with the normal cell. Similarly with a walled cell it is easy to determine the osmotic value at incipient plasmolysis, but

since the cell wall is more or less extensible and is normally stretched, the cell, wall and all, contracts somewhat before plasmolysis begins, and in so doing concentrates itself (Fig. 33). If the shrinkage is considerable it is necessary to correct for this in estimating the osmotic value of the normally distended cell. The volume of the latter is usually estimated by measuring its dimensions in oil.

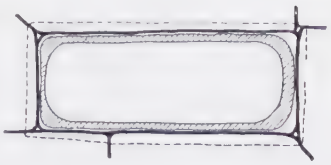

Fig. 33.

3. Turgor Pressure.—In walled cells in their normal state the internal pressure is balanced by the pressure of the wall added to the external osmotic pressure, if any. With a change of medium the membrane stretches or respectively contracts until this equilibrium is again reached. That fraction of the pressure from within which just balances the pressure of the stretched wall is called *turgor* or turgor pressure, and the condition is known as turgidity. Even naked liquid protoplasm may have a slight turgidity owing to the force of surface tension, while in gels the volume elasticity which resists swelling presents analogous features. Turgor is the main cause of rigidity in soft cells and tissues and is also one of the factors in normal cell enlargement. Cambium cells in trees are often under a mechanical pressure of 15 atmospheres or more which they have to overcome in growth. Cells grown in a medium of abnormally high osmotic pressure are usually stunted. They tend however to develop a counterbalancing concentration of their own. Surprisingly high osmotic pressures, between 100 and 300 atm. have been recorded for cells of fruits, of brine organisms and of fungi living in concentrated nutrients.

4. Relation of Cell Volume to Osmotic Value.—In a typical plant cell with large vacuole the volume occupied by the plasmolyzed protoplast is approximately in inverse ratio to the osmotic value of the plasmolyzing solution which of course corresponds to that of the cell itself. The fact that pressure within the cell varies as concentration—that Boyle's law applies—again proves that we are dealing with osmotic pressure as displayed by dilute solutions. In the case of elongated cylindrical cells in which up to rather

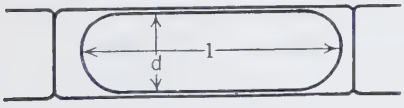

Fig. 34.

extreme plasmolysis the volume of the protoplast is roughly proportional to its length (more nearly to $l - \frac{1}{3}d$, where l = length and d = diameter) one can very quickly estimate the osmotic pressure of an unknown solution. With the aid of a micrometer eyepiece the volume of the cell in the unknown is compared with that in a known solution, and the pressure is obtained by simple proportion. Similarly the osmotic pressure at incipient plasmolysis may be estimated at a single trial. The above is Hofler's "plasmometric" method of comparing osmotic pressures.

In animal cells, however, and in all cells rich in protoplasm or other colloidal material, the osmotic value increases much more rapidly than the volume diminishes, increasingly so as water becomes low. The volume of red blood cells is halved if the concentration of external salt solution is trebled, but thirty-fold concentration barely reduces it to one-third. This is not surprising even if we assume the pressure to be entirely osmotic since the water content of blood cells at normal volume is only 65 per cent, and in concentrated solutions it is the volume (or weight) of solvent rather than of solution that is reciprocal to the pressure. Also swelling pressure, which is probably the main retentive factor in cells poor in water, follows a similar ascending curve with respect to concentration (see page 167).

5. "Suction Force."—Movements of water through tissues are determined by the relative attraction for water of its cells. It is desirable to have some standard of comparison. That which is usually taken is the pressure with which water tends to be absorbed when pure water bathes the cell. This is equal to the osmotic (including swelling) pressure of the cell minus the inward pressure of the wall (when one is present) since the latter tends to squeeze the water out. The resultant has been called the "suction force" (Haftspannung) or "suction pressure" (or "tension") of the cell. Both terms are objectionable but the idea is useful. "Suction force" is most simply measured by determining the external osmotic pressure in which the cell volume remains unchanged. A solution of such osmotic pressure obviously attracts water with the same force as the cell in its normal state. Osmotic values at *incipient plasmolysis* are not always indicative of relative suction pressure since some cells have much more extensible walls than others and shrink more prior to plasmolysis. Certain movements of water through tissues are readily explicable in terms

of a gradient of suction pressure. According to Ursprung and Blum such a gradient exists, e.g. from the central cylinder of a root to the root hairs and from higher to lower leaves of a plant.

Bleeding of plants is also explained by the suction force which develops in the vessels as soon as these are cut open and the mechanical pressure in them is released.

6. Secretion.—Water along with certain solutes—sometimes passes out of cells apparently in opposition to suction pressure. This is found, for example, in glands of animals and plants and in the passage of water into the dead conducting strands in roots. Similarly the endodermis of a root is said to have cells of lower suction tension on either side of it; yet water passes through them. In no one case is the mechanism of this pumping action fully understood. One hypothesis may be mentioned since it postulates local difference in suction pressure on either side of the cell. Lepeschkin has pointed out that if permeability is lower on one side than on the other, the effective suction pressure will also be lower and water as well as solutes will pass out at that side to be replaced by absorption of water at the other side. If the action is to continue the cell must of course *do work* to elaborate more solutes to replace those lost or else to reabsorb them. The above is merely one hypothesis as to its mode of work. In fact the only thing certain is that the cell must expand energy to overcome the pressure gradient. In a later section it will be shown how as one step in the transformation of energy, osmosis may result from electrical forces which reside in cells.

7. Movements Due to Turgor Change.—Most movements of plants are of this nature. Even the slow growth movements are determined by turgor, but the more rapid " variable " or reversible movements are directly due to changes in this respect, and many examples of ejaculatory discharge of spores, etc., also depend on this motive force. The point which deserves a little attention here is the mechanism whereby turgor is modified (dealing only with osmotic pressure at this stage). It may either result from a change in the concentration of osmotic units in the cells or in the permeability of the membranes.

An example of the former is the movement of guard cells. When soluble carbohydrate accumulates in these—through hydrolysis of starch—their turgor goes up, resulting in a change of shape which opens the stoma. By raising the external osmotic pressure we may

reverse the movement. Another possible example is afforded by the movements of *Mimosa*, the sensitive plant. When the plant is stimulated a cushion of cells at the base of and on the under side of the leaf stalk suddenly excretes fluid into the intercellular spaces, causing shrinkage of the cells and bending of the petiole at that point. Gradual reabsorption of the fluid brings about recovery. The suddenness of the turgor change in this case suggests that permeability rather than osmotic pressure is the variable, but the excretion of solutes with the water has not been proved.

8. Rate of Osmosis in Cells.—The speed of such movements as those of *Mimosa* and the rapidity with which substances are known to enter and leave cells (as in the exchange of glucose between blood cells and plasma) are in strong contrast to the very slow approach to equilibrium in our physical experiments on osmosis. The intensity of osmotic action depends, however, not merely on the difference of concentration on two sides of a membrane but also inversely on the thickness of the membrane. Since the physical membranes used are probably 10,000 to 100,000 times thicker than the plasma membrane, and since also subdivision into cells exposes a large surface, the rapidity is not surprising. In a later section it will be shown that osmosis may also be caused by a difference of electrical potential on either side of a membrane. The same argument as to the thickness of the membrane applies in this case; thus small biological potentials may have large significance. Of course conditions at equilibrium are independent of the thickness of the membrane.

CHAPTER IX

IONS AND THEIR DETERMINATION IN PHYSICAL SYSTEMS

The concentration, independent of the nature of the ions in a solution is all that concerns us in dealing with osmotic pressure, but when other physical as well as chemical activities are involved we must consider the concentration of certain particular ions and classes of ions. H- and OH-ions occupy a position of unique importance partly on account of certain peculiar properties of the ions themselves and partly because they are the products of dissociation of water, the commonest and, especially in biology, the most important of solvents. Only about one molecule out of 555 million water molecules is dissociated, but this ratio—small though it is—is very constant unless there is dissolved in the water an electrolyte which itself dissociates either H- or OH-ions, that is, an acid or a base.

I. LAWS OF ELECTROLYTIC DISSOCIATION

1. H-ion Concentration v. Concentration of Titrable Acid.—The total concentration of acid or base in a solution is determined by titration, but this does not tell us the concentration of H- and OH-ions because weaker and stronger acids or bases differ in degree of dissociation. We can neutralize 10 cc. of N/10 HCl and also 10 cc. of N/10 acetic acid by 10 cc. of N/10 NaOH, yet the first acid solution contains 70 times more H-ions than the second. It is the H-ion, not total acid concentration, which controls the chemical and electrical potency of a solution both *in vitro* and *in vivo*. Hence the importance of being able to measure H-ion concentration.

There are two ways of making the measurement and in learning these we shall discover how the concentration of other ions also may be measured and something of what determines ion equilibrium.

2. Dissociation of Weak Electrolytes. Dissociation Constant.—To take the chemical equilibrium (of ions) first, in spite of their electric charge ions seem in this respect to be governed (in large degree) by the same law that applies to other equilibrium reactions: the *mass law*. In its simplest case this law states that when a molecular species A decomposes into two molecular species B and C, the action being reversible, equilibrium is reached when the "active mass" of A bears a certain constant ratio to the *product* of the "active masses" of B and C. Active mass depends on pressure and in dilute solutions this is equivalent, as we have seen, to "concentration." Therefore, for dilute solutions

$$\frac{[A]}{[B] \times [C]} = K,$$

a constant, the double brackets indicating concentration. The actual value of K depends on the affinity of B and C, that is, their tendency to unite to form A and on the temperature. It is called the **affinity constant** of the reaction, and the reciprocal value $\frac{[B] \times [C]}{[A]}$ is called the **dissociation constant** of the substance A. Why this equilibrium law should hold can be understood by considering the relative velocities of the reversible reaction.

$$A \rightleftarrows B + C.$$

The velocity of the decomposition reaction is of course proportional to the pressure or concentration of A, i.e. [A]; the union of B and C to the concentration of B and also of C and, therefore, to the product of [B] and [C].

$$V_1 = K_1 [A] \qquad V_2 = K_2\{[B] \times [C]\}$$

At equilibrium $V_1 = V_2$, therefore $K_1 [A] = K_2\{[B] \times [C]\}$ and

$$\frac{[A]}{[B] \times [C]} = \frac{K_2}{K_1} = K.$$

Applying this law to the dissociation of an acid HA which is expressed as a reversible reaction, thus

$$HA \rightleftarrows H^+ + A^-$$

equilibrium should exist when

$$\frac{[H^+] \times [A^-]}{[HA]}$$

has a certain constant value K_a, the dissociation constant of the acid. ([HA] is the concentration of undissociated acid.)

3. Dissociation of Strong Electrolytes.—Actually the formula is found to hold for weak acids (where K_a has a low value) and for weak electrolytes generally, at least up to a reasonable concentration—because the concentration law fails at high concentrations in non-ionic as well as ionic reactions. With strong electrolytes, however, the formula does not apply even in dilute solutions. It has been demonstrated within recent years that strong electrolytes are probably completely dissociated or practically so and that the apparently incomplete dissociation as calculated from conductivity, osmotic pressure, etc., expresses a restraint that is put upon the ions in general by the residual attraction of oppositely charged ions, just as in more concentrated solutions the attraction between molecules necessitates a modification of the simple Van't Hoff formula for osmotic pressure. By *residual attraction* is meant the attraction which exceeds the repulsion of like charges, owing to the average distance between dissimilarly charged (attracted) ions being less than between similar (repelled) ions.

This explains a certain discrepancy which is found between determinations of ionic concentration made by the various methods, osmotic pressure, conductivity and electrical. The last of these, that with which we are now concerned, is said to measure the " activity " since it does not measure the true concentration of H- and other ions. Its value is not reduced by this changed conception, however, since it is just this "activity" that is of importance and that we wish to know. Nor has it yet become customary to abandon the older terminology; therefore, we shall still speak of " H-ion concentration " and " degree of dissociation." The terms are at any rate truly descriptive when applied to weak electrolytes which exhibit dissociation constants as above defined.

4. Dissociation of Water.—The dissociation of water, a very weak electrolyte, requires special notice.

$$\frac{[H^+] \times [OH^-]}{[H_2O]} = K$$

Since the proportion of undissociated water is relatively large it is practically constant even when acid or base is added. It may, therefore, be transferred to the right hand side of the equation thus:

$$[H^+] \times [OH^-] = K_w$$

This applies to dilute solutions. In concentrated solutions H_2O is not altogether constant. K_w is termed the dissociation constant of water. At 22° C. it is $\frac{1}{10^{14}}$. $[H^+]$ and $[OH^-]$ are each $\frac{1}{10^7}$ normal. The addition of acid increases the number of H-ions and decreases that of OH-ions in such a way that the product remains 10^{-14}. Similarly, a base increases the ratio of OH-ions without altering the product. It is thus unnecessary to mention both OH and H and it is customary to describe even alkaline solutions in terms of H-ion concentration.

5. Meaning of *p*H.—Just as the normality of an acid solution depends on the number of grams of *ionizable H* (normal solution = 1 gram per liter), so the normality of H-ions $[H^+]$ depends on the number of grams of *ionized H*. Since the values of [H] (10^{-7}) are cumbersome in calculation and in curve plotting and since also it happens that the negative logarithm of $[H^+]$ ($-\log [H^+]$ or $\log \frac{1}{[H^+]}$) is first obtained in calculating $[H^+]$ from potential measurement, this value is most commonly used under the name of *p*H. Thus an $[H^+]$ of 10^{-7} equals a *p*H of 7. In a scale of *p*H values, 1, 2, 3, etc., each successive number in the scale represents 1/10 of the number of H-ions that the previous one does.

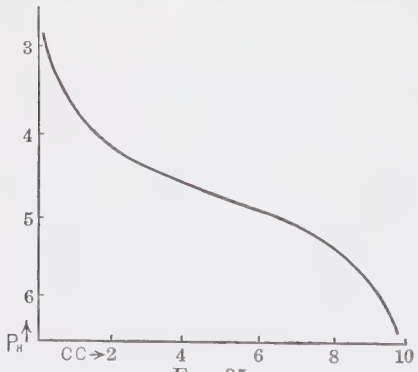

Fig. 35.

6. Titration Curves. Buffer Solutions.—Certain very important relations are brought out by a consideration of how H or rather *p*H varies during titrations of acid or base— especially when one of the reacting substances is weak electrolyte. Figure 35 shows how the *p*H varies when 10 cc.

of N. acetic acid is titrated with N NaOH. The curve is sigmoid (S-shaped). The *p*H changes rapidly at the beginning and near the neutralization or end-point, more slowly at intermediate stages, and least of all when the acid is half converted to salt or when acid and salt are present in equal normal concentration. Here we must introduce a new and important term. A solution is said to act as a **buffer** in proportion as it resists change of *p*H with addition of acid or base. A mixture of acetic acid and Na acetate is such a buffer, especially when the mixture is in equal proportions of normality, as shown by the flattening out of the curve in its middle region.

Other features of titration curves and buffers are brought out most simply from theoretical considerations. To get an equation involving *p*H we write our original equilibrium formula in the shape

$$\frac{1}{[H^+]} = \frac{[A^-]}{K_a[HA]}$$

from which

$$\log \frac{1}{[H^+]} \quad \text{or} \quad pH = \log \frac{1}{K_a} + \log \frac{[A^-]}{[HA]};$$

K_a is a constant; $\log \frac{1}{K_a}$ for acetic acid equals 4.74. An approximate value for $\frac{[A^-]}{[HA]}$ is got from the following considerations. Since a weak acid like acetic is only slightly dissociated while salts even of weak acids are almost completely dissociated, the acetic anions A^- are practically all derived from the salt and the concentration of undissociated acid is approximately equal to that of total acid. Therefore $pH = 4.74 + \log \frac{[Salt]}{[Acid]}$ (approximately). Two results emerge from this equation which experiment will confirm:

(1) The *p*H of such a solution depends mainly on the proportion of salt to acid and not greatly on the concentration. In other words, another feature of buffer solutions is that their *p*H is little affected by dilution.

(2) At the point of maximum buffering when [salt] = [acid] the $[H^+]$ is numerically equal to the dissociation constant of the acid $\left[\text{for since } \log \frac{1}{[H^+]} = \frac{1}{K_a} + 0, \text{ therefore } [H^+] = K_a\right]$.

To get good buffering at any required H-ion concentration we, therefore, take a weak acid whose dissociation constant is of nearly the same numerical value as the required $[H^+]$ and either mix it with an equal normal concentration of salt or half titrate it with a strong base. Examples are a mixture of acetic acid and Na acetate, of primary and secondary phosphate, of boric acid and borax.

The practical importance of what we have been considering lies in two facts:

(1) The colour change of **indicators** (which provides one means of measuring H-ion concentration) is the result of a transformation of an acid to a salt or a base to a salt, or vice versa as the case may be, which transformation follows much the same course as in the example we have studied.

(2) Buffer solutions are used whenever a constant *p*H is demanded, for instance, for colour standards with the indicator method of measuring *p*H, for culture media, perfusion fluids, etc.

7. Theory and Use of Indicators.—Indicators may be regarded as weak acids or bases (or salts of these) of which the dissociated (organic) ion has a different colour from the undissociated molecule, or may be coloured as compared with a colourless molecule. Actually the colour change is the result of a rearrangement of atoms in the molecule; but since one of the isomers is a strong electrolyte, it comes to the same thing practically as if we were dealing simply with a case of ionic equilibrium. The main difference is seen in the appreciable time that is sometimes required to complete the colour change. Tautomeric transformations are slow compared with ionic reactions. If the indicator is a base it dissociates in a relatively acid solution; if an acid, in an alkaline solution. If we set up a long series of tubes to cover a wide *p*H range, we should find that toward the acid end of the series an added indicator assumes one colour and towards the alkaline end another colour, while in between there is a narrow transition zone (not more than 2 *p*H) with intermediate shades which represent a mixture of the two colours. The position in the *p*H scale of this transitional zone varies with the indicator. Only with some, such as neutral red, does it include the neutral point. Within this zone the indicator tint accurately tells us the *p*H, assuming that we are provided with a standard by which to judge it. On either side

of this zone the colour remains unchanged throughout the rest of pH range and, therefore, tells us nothing as to the exact position on the scale. Thus to cover the whole pH range a series of indicators with different but overlapping transformation zones is required, and that one discovered by trial which shows a transition tint in the solution whose pH has to be determined.

It is important for accurate work that in comparing the tint of an indicator at any stage of transformation with its colour in standard solutions, the concentration of indicator and the diameter of test-tubes shall be the same in both cases. In using indicators to discover the pH of cells under the microscope it is very difficult to arrange comparable colour standards and the apparent quality of the tint is found to vary considerably with the concentration of dye in the cell. In such work it is usually better to depend mainly upon the end colours of different indicators of approximate ranges. Thus if the vacuole of a cell shows yellow with Methyl Red it is above pH 5.6 and if it shows yellow with Brom Cresol Purple it is below pH 5.8. These limits are usually as narrow as need be in view of other sources of error.

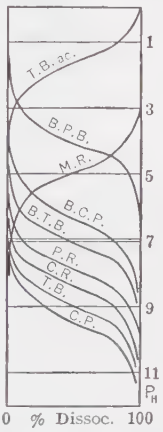

Fig. 36.—(After Clark.) pH range of some common indicators. (Clark and Lubs.)

T. B. Thymol Blue. *ac*: acid range
B. P. B. Brom Phenol Blue
M. R. Methyl Red
B. C. P. Brom Cresol Purple
B. T. B. Brom Thymol Blue
P. R. Phenol Red
C. R. Cresol Red
C. P. Cresol Pthalein.

Figure 36 shows roughly the dissociation curves of a number of indicators. It is in the middle, the flattest part of its curve, where the colour changes most rapidly with the pH, that an indicator is most sensitive. The curves of acidic indicators slope in one direction (higher pH = higher dissociation); those of basic indicators in the opposite direction. There is nothing in the colour change to tell us whether we are dealing with acidic or basic dyes; but in estimating the pH of colloidal solution and of cells it is of importance to discriminate, since an adsorbed

indicator fails to serve its purpose. Usually acidic dyes are least adsorbed but on the other hand they are often unable to penetrate living cells.

In passing we may add a note as to choice of indicators in titration. When both acid and base are strong the salt produced (as NaCl) is neutral, and in absence of free acid or base the *p*H is about 7. Therefore an indicator whose transformation point is near *p*H 7 may be used—such as neutral red. When the acid is weak the salt (as Na acetate) is alkaline owing to hydrolysis, and an indicator whose transformation point is on the alkaline side of neutrality should be used—such as phenolphthalein. When the base is weak the salt (as NH_4Cl) has an acid reaction for the same reason and an indicator which changes in a weakly acid medium should be employed—such as methyl red.

II. THEORY AND USE OF POTENTIOMETER IN DETERMINING ION CONCENTRATION

The indicator method of determining *p*H is valuable on account of its speed and simplicity. When there is much protein or other colloid or even much salt in the solution the ionic equilibrium of indicators is affected thereby and the results show **protein error** or **salt error** as compared with the hydrogen electrode method which is in all cases taken as the standard. It is desirable, therefore, to understand the latter method also, whether we use it commonly or not, especially since the concentration of other than H-ions may be determined in a similar fashion. The method depends upon the effect that ion concentration has upon the potential difference between the solution and a suitable electrode.

1. Electrode Potential.—A metal immersed in a solution of its ions shows a potential difference toward the solution which depends first upon the nature of the metal and second on the concentration of its own ions in solution—but not on the concentration of any other ions. Third, temperature has a slight influence. Ions tend to pass from the metal into the solution with a certain force. The reverse also tends to happen with a force which varies with the partial pressure of the ions in the solution. The former force was ascribed by Nernst to what he called the "electrolytic solution pressure" of the metal. With a certain osmotic pressure of ions in solution the "solution pressure" should

be in equilibrium. This value has been estimated for the different metals and also for elements in general. In some cases it is so high and in others so low as to have no physical significance (Zn = 9.9×10^{18} atmospheres, palladium 1.5×10^{-36} atm.). Thus, metals like Zn are always + and noble metals like Pd, Hg, and Ag always −. The actual values of the electrolytic solution pressure of the various metals does not greatly concern us because the variable factor in measuring ion concentration is not the nature of the electrode but the concentration of ions. Qualitatively it is obvious that the higher the concentration of the metallic ions in a solution the more positive (or less negative) will be an electrode of the metal immersed in it. Nernst showed that the electrode potential can be quantitatively related to the pressure of ions in solution and the hypothetical " solution pressure " of the metal

$$E = \frac{RT}{nF} ln \frac{K}{p}$$

where R = gas constant;
F = faraday (amount of electricity associated with 1 gram-equivalent);
ln = natural logarithm to base *e*;
K = solution pressure of electrode metal;
p = osmotic pressure of the ions.

2. Concentration Chain.—If two solutions are connected through electrodes of the same metal, E_1 and E_2 being the respective electrode potentials and p_1 and p_2 the osmotic pressures of the common ion, the potential difference (E) between the two solutions is as follows:

$$E = E_1 - E_2 = \frac{RT}{nF} ln \frac{p_1}{p_2}$$

(The derivation of these formulae will be found in textbooks of Physical Chemistry.)

If these two solutions of different concentration are brought directly into contact to complete the chain and allow a current to flow, there is likely to be a certain potential difference at the junction caused by unequal speed of diffusion of cations and anions respectively (diffusion potential). To eliminate this a bridge of saturated KCl is interposed, which, owing to the relatively high

concentration of its ions, swamps the effect of the dilute electrode solutions (Fig. 37). There is thus a practically equal and opposite potential diffusion at the two liquid junctions and this also is very small since K and Cl ions migrate at almost identical rates. In dilute solutions Concentration may be substituted for Pressure and $E = \frac{RT}{nF} ln \frac{C_1}{C_2}$. This is the fundamental equation for all concentration chains. (The values of the constants are given under the discussion of the hydrogen electrode.) The condition *sine qua non* for this equation to hold is that both electrodes be reversible. As already mentioned, this is the case with a metal electrode in a solution of its salt but it is not confined to this type.

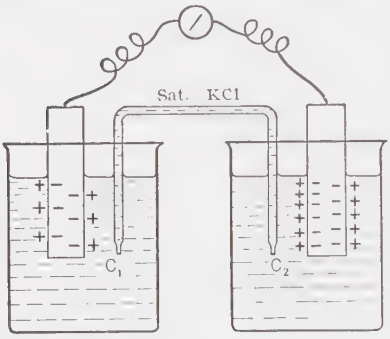

Fig. 37.

3. Types of Electric Cell Commonly Used in Biological Work.—The three types of electrode which are most used in biological work are the calomel electrode, the hydrogen electrode and the quinhydrone electrode. Two of the same kind or two different ones may be connected to form a cell.

(*a*) *The Calomel Electrode* consists of an electrode of Hg in contact with a solution of calomel (most commonly saturated). HgCl is not only reversible for Hg^+ ions but also for Cl^- ions. Owing to the low dissociation of HgCl the relation between $[Hg^+]$ and $[Cl^-]$ in a concentrated HgCl solution is similar to that between $[H^+]$ and $[OH^-]$ aqueous solutions, namely,

$$\frac{[HgCl]}{[Hg^+] \times [Cl^-]} = K.$$

Since [HgCl] (the concentration of undissociated HgCl) is practically constant, $[Cl^-]$ varies inversely as $[Hg^+]$, and the potential depends on $[Cl^-]$. Cl ions being common in nature and nontoxic, the calomel electrode is very useful in measuring biological E.M.F's For this purpose two calomel electrodes are used to form a concentration chain with reference to Cl^-. On account of

its stability it is also used in conjunction with the hydrogen electrode in the determination of $[H^+]$.

(*b*) *The Double Hydrogen Electrode.*—By taking advantage of the fact that certain noble metals adsorb hydrogen gas, we can construct an electrode which is reversible to H-ions and is called a hydrogen electrode. Platinum coated with platinum black—to increase surface—is the usual adsorbent. The solution pressure of the electrode depends on the gas pressure of hydrogen. With *two hydrogen electrodes*, therefore, the pressure of hydrogen must be the same at each if the potential is to depend simply on the H-ion concentration of the solutions. This is easily effected by bubbling H_2 at atmospheric pressure through the two electrode solutions. As with other concentration chains,

$$E = \frac{RT}{nF} \, ln \, \frac{[C_1]}{[C_2]} = \frac{RT}{F} \, ln \, \frac{[H_1]}{[H_2]}.$$

(Hereafter [H] is printed for $[H^+]$.)

R is the gas law constant: RT = PV. In electrical units (volt-coulombs) its value is 8.313. F is the faraday = 96,450 coulombs; n for H is unity; *ln* can be converted into $\log_{10}$ by dividing by 4343. Hence $E = 0.00019837 \; T \log \frac{[H_1]}{[H_2]}$.

For convenience we shall call the value 0.00019837 T, the *factor F*. The value of *F* at 18° C. (273° + 18° = abs. T) is 0.0577; and if E is expressed in millivolts instead of volts *F* becomes 57.7. For each degree rise of temperature it increases 0.02. Thus

t Centigrade	*F* When E is Expressed in Millivolts	E sat. Cal. Electrode Millivolts
16	57.3	251.7
18	57.7	250.3
20	58.1	248.8
22	58.5	247.5

Diff. 0.67 per 1°

If we have two hydrogen electrodes, one dipping into a solution of $[H_1]$ hydrogen-ion concentration and the other of $[H_2]$, the first electrode will be positive if $[H_1] > [H_2]$.

According to the condensed formula

$$E \text{ (in millivolts)} = F \log \frac{[H_1]}{[H_2]}$$

or

$$\frac{E}{F} = \log [H_1] - \log [H_2]$$

or since

$$-\log [H_1] = p\text{H of solution 1}$$

and

$$-\log [H_2] = p\text{H of solution 2}$$

$$\frac{E}{F} = p\text{H}_2 - p\text{H}_1$$

Therefore, if we know $p\text{H}_1$, and measure E, $p\text{H}_2$ is easily calculated. Commonly a standard acetate buffer (a solution containing acetic acid and Na acetate in equal concentration—usually 0.1 N) is used as a solution of known pH, namely 4.63, practically unaffected by ordinary range of temperature. If the standard acetate solution is more acid than the unknown its electrode will be positive, and observed $E = F \log \frac{[H_{ac}]}{[H]}$ where [H] is the H-ion concentration of the solution to be tested. If the standard acetate is less acid its electrode will be negative and observed $E = F \log \frac{[H]}{[H_{ac}]}$. In the former case (electrode of standard +):

$$p\text{H} = 4.64 + \frac{E}{F}$$

In the latter case (electrode of unknown −):

$$p\text{H} = 4.63 - \frac{E}{F}$$

(c) *Hydrogen Electrode with Calomel Electrode.*—It is more common to replace the standard hydrogen electrode by a calomel electrode, especially when—to avoid carrying off dissolved CO_2—a closed, shaking electrode vessel is used instead of a bubbling one. The formula then used is derived as follows: If the standard electrode were a hydrogen electrode normal with respect to H-ions

E would equal $F \log \frac{1}{[H]}$ since normal [H] = 1. But the above E = observed E.M.F. with a calomel electrode *minus* the difference between the calomel and the normal hydrogen electrode. The E.M.F. of the normal hydrogen electrode is defined as zero and the potential of the calomel electrode at various temperatures is given in the table on p. 117.

$$\text{E.M.F. observed} - \text{E.M.F. calomel electrode} = F \log \frac{1}{[H]}$$

$$p\text{H} = \frac{\text{E.M.F. (obs.)} - \text{E.M.F. (cal. elec.)}}{F}$$

$$\text{at } 18^\circ \text{ equal to } \frac{\text{E.M.F. obs.} - 250}{57.7}$$

The hydrogen electrode method of measuring *p*H, though superior to the indicator method in sensitivity and in freedom from protein and salt error, has several disadvantages. It demands cumbersome equipment, takes long to set up when not in regular use, is not portable and can hardly be arranged to give reliable results when—as is frequently the case in biological fluids—acidity is in any large measure determined by CO_2.

(*d*) *The Quinhydrone Electrode* (*Double or Single*).—The quinhydrone electrode, which retains most of the advantages of the electrical method, also escapes the above drawbacks of the hydrogen electrode. Besides being accurate, it is simple, portable, easy to set up and easy to use. Unfortunately, it can not be used with accuracy above *p*H 8 or at most *p*H 8.5, but most biological fluids are of lower *p*H than this.

If two unattackable electrodes dip into a mixed solution of quinone (Q) and hydroquinone (QH_2) and a current is passed through, QH_2 is oxidized to Q at the positive electrode and Q is reduced to QH_2 at the negative one. The process is reversible, and by setting up a chain of two such electrodes we get an oxidation-reduction cell capable of producing a current. It is possible to picture the quinhydrone electrode simply as a hydrogen electrode in equilibrium with the hydrogen liberated in the reaction

$QH_2 \rightleftarrows Q + H_2$. By the mass law this hydrogen pressure varies as the ratio of the concentration of QH_2 to that of Q.

$$[H_2] = K \frac{[QH_2]}{[Q]}$$

Therefore, if the ratio of $[QH_2]$ to [Q] is kept the same in both electrode solutions, the E.M.F. of the chain depends as before simply on the *p*H of the respective solution as follows:

$$\frac{E}{F} = pH_2 - pH_1$$

(See under double hydrogen electrode.)

A more general interpretation of the mechanism of oxidation-reduction potential, which covers cases where no hydrogen is involved (as in a ferric-ferrous cell) may be found in Clark's or Michaelis's textbooks. Suffice it to say here that the same theoretical formula may be derived.

The convenience of the quinhydrone electrode is enhanced by the ease with which the ratio of QH_2 to Q is kept constant. The two combine in equal molecular proportions to form a not very soluble crystalline compound, quinhydrone, which again dissociates in aqueous solution. Thus, if we dissolve some quinhydrone we automatically obtain a solution in which quinone and hydroquinone are in equal concentration. The quinhydrone electrode may also be used along with the calomel electrode. About the only advantage is that the latter is always positive to the test solution, which saves trial for polarity—but the double quinhydrone electrode saves correction and is simpler to understand.

5. Measurement of Potential.—The principle of the potentiometer is the compensation of the unknown potential by an equal potential which can be easily measured. The latter is graded by varying a known resistance. The essential arrangement is illustrated in the diagram (Fig. 38). *OP* is a wire of uniform resistance along which one can obtain a regular fall of potential by passing a current of constant strength through it from the battery *B*. One terminal of the unknown *X* is tapped in at the end of the wire which is connected with the same pole of *B* (say +). The potential at *O* is now that of the + pole of *X*. The other terminal bearing the

relative potential of the negative pole of X is tapped in at some point L on OP. If it is relatively far from O and meets with a lower potential than its own, then current flows from it resulting in a current along OXL. If it meets with a higher potential, current will flow in the reverse direction LXO. If it meets with the same potential, no current passes. The null point is commonly found by means of a galvanometer inserted somewhere between O and L. At equilibrium the difference of potential between the two terminals of the unknown X is therefore the same as the difference of potential between O and L. But the fall of potential between O and L is simply OL multiplied by the fall per unit length, which we know from preliminary graduation (see below).

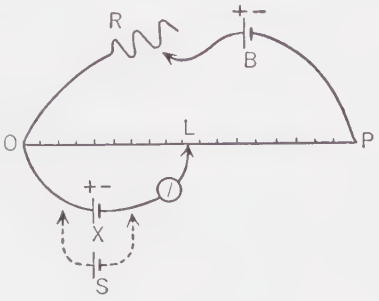

Fig. 38.

In order that the gradient of potential along OL shall always be the same once the wire has been graduated, a resistance is put in the current from B so that as the battery runs down the resistance may be correspondingly reduced to give a constant current and, therefore, constant E.M.F. $\left(C = \frac{E}{R}\right)$ through the wire. This is checked and corrected from time to time by switching a standard cell (S) in place of the unknown X and regulating R until the proper E.M.F. of the cell is secured. The same arrangement is used to graduate the wire originally. Although it is instructive to make and use a simple potentiometer on the above model, using a simple meter bridge to correspond to OP, a manufactured instrument is more convenient for regular work.

CHAPTER X

IONS, PARTICULARLY H-IONS, AND THEIR DETERMINATION IN CELLS

1. The Electrolytic Environment of Cells.—A fairly definite and constant electrolytic environment is one of the necessities of vital activity. Practically all cells to be active must be bathed by water containing mineral salts, and, in varying degree, they are sensitive to the character of these salts. The cells of the animal body are particularly definite in their requirement both as to the osmotic pressure of the surrounding medium and to the nature and proportions of the ions present. This medium is the serum of the blood-lymph system, which bathes all the cells of the body and which by elaborate regulation is kept remarkably constant.

The resemblance between the ionic content of this " internal environment " which animals carry around with them and that of sea water is noteworthy. The proportions of Na, Ca and K is shown below.

	Serum	Sea Water
Na	100.0	100.0
Ca	2.58	3.84
K	6.69	3.66

Sea water is more concentrated and contains Mg in addition, yet if diluted to the osmotic pressure of blood it forms a very effective " physiological solution," that is, it maintains cells in a normal state.

From the evolutionary standpoint the explanation of this similarity between serum and sea water is the marine origin of life. Animals in passing to a land life are supposed to have carried their ancestral environment with them. The lack of Mg may be a subsequent modification or it may, as Macallum suggests, indicate a different composition of the pre-Cambrian seas at the time when the terrestrial migration began.

Plant cells differ from animal cells in being adapted to life in

" fresh " water. Equally with animal cells they are dependent on water and, as plants in development raise themselves above the earth, they lift up also their aqueous environment. At the top of the tallest tree, " in the eye of the sun," the cells are constantly laved by water. Plant cells contain within themselves in the central sap vacuole a solution which as regards osmotic pressure resembles the external environment of animal cells but not their own. The lack of osmotic balance which thus exists between the inside and outside of plant cells is rendered possible as we have seen by the presence of rigid cell walls. A great latitude of osmotic pressure in the environment can thus be tolerated by these cells.

Plants have to endure vicissitudes in the composition as well as concentration of their medium so that, as might be expected, we find them more tolerant as to ionic ratios in that medium. Thus, while the heart of a vertebrate animal will beat only when perfused by a solution of very definite composition as regards ratios of Na, K, Ca and also H (a " Ringer-Locke " solution is NaCl . . . 0.9, KCl . . . 0.042, $CaCl_2$ . . . 0.024, $NaHCO_3$ . . . about 0.02 [glucose about 0.2] and water up to 100), plants grow equally well in decidedly varied culture media, varied both as to ion balance and *p*H. Nevertheless the difference is only one of degree. Plant cells, too, demand a certain ionic balance. Their requirement in this respect is to be distinguished from their needs with regard to nutrition. Certain elements including K, Ca and Mg are required to build up protoplasm and must be present in some slight concentration if growth is to continue, but selective absorption dispenses with the necessity of their being present in any definite ratio. The need for a certain ratio of monovalent to divalent cations and of K to Na, etc., depends largely on the physical effect of these types of ion on protoplasm and especially on the plasma membrane. This influence may be exercised by non-nutritive as well as nutritive ions. Strontium, for example, may for a time take the place of calcium. How these ions act will be discussed under Colloids.

The estimate of the concentration of most ions whether in cells or cell media can be made only by chemical analysis. Cl^- in the medium or cell sap can be measured with the aid of the calomel electrode. H^+ requires special notice.

2. Activity of H- and OH-ions.—Of all ions the hydrogen and hydroxyl are of greatest importance in physiology as well as

in chemistry on account of both their commonness and their activity. As an example of their activity, a frog's heart which beats normally in a perfusion fluid containing a balanced mixture of ions at the neutral point dies if the H-ion concentration is raised by only 1 millionth of a gram-ion (10^{-6} N); or again, the respiratory center in the brain of higher animals is more sensitive to H-ion changes than any artificial indicator. The H-ion concentration of the blood is bound up with the tension of CO_2 in the blood and this with the tension in the alveoli of the lung; therefore, an increase of CO_2 concentration in the gas of the lung spaces, by as little as $\frac{1}{5}$ of 1 per cent will double the respiration. The effect on respiration rate of burying one's head under the blankets illustrates the point.

CO_2 concentration is modified not only by respiration but in plants to a greater degree by photosynthesis. In plants as in animals there is a regulatory mechanism in the ventilatory system, and, though mechanically utterly different from that of higher animals, it is governed by the same chemical agency, namely, H-ion concentration. Other relations of life to H-ion concentration will be mentioned in dealing with colloids, but enough has been said to show the importance of being able to measure accurately the concentration of H-ions not only in the surrounding medium but in the cells themselves. The general methods of determining *p*H in physical systems have already been described. It only remains to point out special technique and precautions which the circumstances demand in dealing with cells.

3. Determination of H-ion Concentration in Cells and Body Fluids. (*a*) *p*H *of Protoplasm.*—The only method that can be regarded as giving reliable results is that of microinjection of indicators, except under abnormal circumstances. Protoplasm itself cannot be stained by dyes which are taken up from the surrounding medium. Such dyes pass into vacuoles or granules, which show a quite different reaction from the ground substance of the cytoplasm. Basic dyes when injected tend to behave in the same way, that is, gradually to become concentrated in inclusions of the cell. If sufficiently dilute, however, they remain in the cytoplasm long enough to permit observation. Acidic dyes remain homogeneously dispersed in the protoplasmic matrix. The common impermeability of the plasma membrane to this class of dyes is a help rather than a hindrance because it prevents

their escape. They are also much less toxic than basic dyes. Thus altogether the best results are obtained by microinjection of acidic indicators of suitable range such as phenol red. By this means it is shown that the *p*H of cytoplasm is very constant, being about 6.8 in most if not all kinds of cells so far tested. The nucleus is more alkaline—about *p*H 7.5. It also appears that the cytoplasm is well buffered. Injection of acid or alkali produces only a momentary and local change of reaction unless the amount injected is so great as to cause irreversible injury. Salts of polyvalent cations when injected have the same effect as acids on the intracellular *p*H. A transitory flash of the acid colour is induced at the locus of injection. Acid or alkali penetrating from the exterior have still less effect. They fail to produce any noticeable change in the protoplasmic *p*H unless the concentration is high enough to be injurious. (It is probable, nevertheless, that, due to the effect of penetrating acid and alkali on enzyme action within the cell, a certain internal variation in equilibium with the environment must take place in response to changes outside.)

(*b*) *p*H *of Vacuoles.*—Vacuoles are generally more acid than the cytoplasm, sometimes to an extraordinary degree. In some fruits, such as limes, the acidity may be as high as *p*H 1.7. As a rule, however, cell sap lies within the colour range of methyl red—about *p*H 5.

Unlike the cytoplasm, the vacuolar sap is poorly buffered. Its reaction varies notably with metabolic activity and under the action of penetrating reagents. Measurement of the *p*H of the vacuole can be accomplished by allowing indicators to be taken up. As a rule only basic indicators accumulate. Such accumulated dyes are often demonstrably adsorbed by colloidal material in the sap. In this state they are inaccurate as indicators although they may serve to demonstrate changes of *p*H. Highly basic dyes such as neutral red are more adsorbed and less indicative of *p*H than a weakly basic or ampholytic dye such as methyl red.

Acidic dyes generally remain in solution in the sap and serve as true indicators. The trouble with these, using the penetration method, is to obtain adequate depth of colour. Sometimes this can be attained by immersing the cells in a strong solution of dye for a time and then transferring to a colourless medium for observation. Precautions to be observed are mentioned in a later paragraph. Many cells, however, are practically impermeable to acidic

dyes and can be stained only by microinjection. In rare instances, for example, *Valonia* and *Nitella*, the "cells" are so large that an appreciable quantity of sap can be obtained by simply cutting the cells across and squeezing them. The crushing method as applied to tissues in general is very unreliable for the following reason.

(*c*) *Acid of injury.*—An important discovery resulting from microinjection technique is the production of "acid of injury." Very slight and even local injury to a cell causes the cytoplasm in the affected region to become temporarily more acid than normal—up to about *p*H 5.5 is the usual extent of the change. This fact stultifies observations of *p*H which involve crushing of cells.

(*d*) *p*H *of Body Fluids.*—Measurement of the *p*H of blood plasma is more a physical than a biological problem. Any of the ordinary physical methods may be applied with a few necessary precautions which need not be gone into here. One must, for example, take count of the fact that CO_2 is an important factor in determining the *p*H of body fluids and the gas should not be allowed to escape if accurate results are required.

4. Biological Buffers.—Constancy of reaction is a feature both of cells and body fluids. This is maintained by a double type of mechanism: (1) elimination of the acid products and (2) buffer action. The latter takes care of the effect of acid before it can be eliminated. In cells, the principal buffers are the phosphates; and in blood the carbonates. Proteins assist to some extent in both. Both carbonates and phosphates are most effective around the neutral point. Taking the carbonate system by itself for simplicity let us estimate how efficient the buffering is.

$\frac{[H^{\cdot}] \times [HCO_3']}{[H_2CO]}$ is a constant $= 3 \times 10^{-7}$ (the dissociation constant of carbonic acid).

Transposing,

$$[H^{\cdot}] = \frac{[H_2CO_3]}{[HCO_3']} \times 3 \times 10^{-7} = \frac{[CO_2]}{[0.8\ NaHCO_3]} \times 3 \times 10^{-7}$$

since $[H_2CO_3]$ is practically equal to $[CO_2]$, and $[HCO_3']$ is practically all derived from $NaHCO_3$ and $= 0.8\ [NaHCO_3]$ the carbonate in blood being 0.8 dissociated.

We can calculate, therefore, that if

$$[H^{\cdot}] \text{ is } 1 \times 10^{-7}, \quad \frac{[CO_2]}{[NaHCO_3]} = \frac{1}{3.75}$$

and if

$$[H^{\cdot}] \text{ is } 0.5 \times 10^{-7}, \quad \frac{[CO_2]}{[NaHCO_3]} = \frac{1}{7.5}$$

That is, the ratio of CO_2 to bicarbonate must be doubled to produce such a minute increase of H-ions as $\frac{1}{20{,}000{,}000}$ N. The equilibrium between CO_2 and carbonates is important to life not only as displayed in the blood of higher animals but also in the whole surface of the ocean where it probably does more to maintain a uniform concentration of CO_2 in the atmosphere than does the biological balance between respiration and photosynthesis. Any reduction of CO_2 in the atmosphere at once releases some of the immense CO_2 reserves stored up in the bicarbonates and carbonates in the seas of the world.

5. Determination of *p*H of Tissue Cells in Situ.—An estimate of intracellular *p*H obtained by staining cells after they have been removed from their normal position is frequently liable to be far from correct—even if the cells are not injured in the process—because the environment has been changed. The vacuolar sap of plant cells, for example, is poorly buffered and changes markedly with variations in CO_2 concentration. The CO_2 concentration inside a cell very quickly comes to equilibrium with whatever medium is outside. It is, therefore, necessary to stain the cells while still in the plant. For this purpose indicators can be brought to the cells in various ways. One is to place cut shoots in the dye solution. Acidic dyes ascend readily with the transpiration current and in many cases enter the living cells. Basic dyes are retarded by adsorption on the walls.

Another method is that of injection of dye into the intracellular spaces. This can be procured by pressure, suction, or centrifuging, applied to leaves or portions of stem in the dye solution. From the intracellular spaces the dye commonly passes into the cells. To observe the colour of the cell sap it is usually necessary to make sections and examine quickly. The general tint of the whole tissue can also be observed by the naked eye, and is found to vary

with metabolic activity. Thus leaves which are yellow with brown cresol purple in the dark become purple or bluish in the light because photosynthesis reduces the CO_2 concentration.

It is easy to bring dyes to the cells of the animal body by injection in the blood stream. As already mentioned, however, not much can be learned about the protoplasmic *p*H in this way, because basic dyes are stored away in cell inclusions and acidic dyes are taken up only by exceptional cells, such as the phagocytes and kidney cells.

6. Potentiometric Determination of *p*H in Cells.— Minute hydrogen and calomel electrodes have been constructed for insertion into cells. Results obtained for the cell sap agree with indicator determinations, but there are too many complicating factors to allow the protoplasmic *p*H to be recorded in this way.

The Importance of Electricity in Vital Phenomena

Whenever a muscle contracts, a gland secretes or a nerve conveys its message, electric currents flow. Even continuous currents of low intensity are said to circulate in growing plants. Cells deserve their name in the galvanic sense more generally than in the meaning with which Robert Hooke first applied the term. Every cell is a battery and its periodic activities are attended by a discharge. Whether these bioelectric changes are of the very essence of life or only incidental to other more fundamental processes is still an open question, but at least they prove to be of great value as a clue to hidden activities of the body and of the cell. Some knowledge of the origin and significance of these bioelectrical phenomena is therefore desirable for the general physiologist.

CHAPTER XI

ELECTRIC POTENTIAL AND ELECTRIC CURRENTS IN PHYSICAL (NON-METALLIC) SYSTEMS

For an electric current to flow there must be potential difference between two regions. Let us first consider how this may arise in physical systems such as are possibly duplicated in living cells.

I. CONDITIONS FOR EXISTENCE OF POTENTIAL DIFFERENCE

The origin of the E.M.F. of metal electrodes has already been discussed. Obviously the potential of living cells and tissues must have some other origin. The possibility that a separation and transfer of electrons may occur in protoplasm will be referred to later, but undoubtedly the more probable and principal cause of bioelectric potential is the separation not of electrons simply but of ions. There are various mechanisms by which cations and anions may be separated in space.

1. Diffusion Potential.—Unequal speed of migration of the respective ions may produce a slight separation wherever there is a diffusion front (Fig. 31, p. 79). The potential gradient which results is merely transient and under the conditions which obtain in cells would be quite small. It is only at phase boundaries and across membranes that potential differences (P.D's) of the value commonly found in living cells can be produced.

2. Phase Boundary Potential.—The condition for an E.M.F. at a non-metallic phase boundary is the presence of an electrolyte whose ions are unequally partitioned between the two phases. Unequal solubility, in a wide sense of the term, is probably the main cause of the unequal distribution, but adsorption at the phase boundary may also modify it. The electrolyte may be a third substance in true solution, for instance, an organic acid distributed between oil and water, the anion being attracted preferably to the oil, the cation to the water. Or it may arise

from one of the phases itself as when a colloid like soap or mastic dissociates in contact with water the inorganic (Na or H) ions at the surface going into solution, the organic anion remaining attached to the body of the particle. We must suppose, in order that the potential may be transmitted throughout the solid phase, that as a result of imbibition of water there is some dissociation in the interior as well as at the surface—a condition analogous to solution of an electrolyte in the phase. In both cases conditions near the interface are different from those within the body of either phase. On the non-aqueous side of the interface there will be an excess of anions and on the aqueous side an excess of cations—held there by mutual attraction and forming the so-called ionic "double layer."

Adsorption of ions which is another cause of the formation of a double layer is found to influence interphase potential in some cases, in others not at all. A species of ion which is unable to enter both phases is unable, whether adsorbed or not, to influence directly the potential difference between them; but it may do so indirectly by displacing from adsorption other ions which are common to the two phases.

3. Electrokinetic Potential.—It is, however, mainly within a very thin layer on the aqueous side of the interface that distribution of charge is influenced by adsorption from the water. In virtue of this influence, adsorption has a profound influence on another kind of potential known as *electrokinetic* (so called because it is displayed during the movement of one phase past the other). This potential, as we shall see, plays a far larger part in the physical chemistry of protoplasm than does phase boundary potential. The physiologically important effect of electric charge on surface tension and on colloidal stability is a matter of electrokinetic potential. This potential is most directly displayed and measured in the phenomena of electrosmosis and electrophoresis. If the two poles of a battery are placed in vessels connected by a capillary tube, or by multiple tubes as in a porous membrane, the liquid in the tube if it bears a charge relative to the wall flows toward the pole of opposite sign. This is **electrosmosis.** The ions in the movable side of the double layer migrate as in electrolysis and carry adhering liquid with them. In **electrophoresis** (or kataphoresis) charged particles suspended in a liquid move under the same influence. The two cases are thus reciprocal.

With certain assumptions the potential between the moving and non-moving bodies can be calculated from the rate of movement in a known gradient of potential, directed at right angles to the phase boundary force. It is found to be less than the phase boundary potential measured as E.M.F. A more striking divergence is seen in the fact that adsorption of ions may reduce and even reverse the electrokinetic potential without affecting the other. The explanation of these discrepancies seems to be: (1) that owing to thermal agitation, etc., the double layer is diffuse or statistical rather than precise and may thus be several millimicrons across; and (2) that a thin layer of liquid of thickness δ adheres to the solid wall (Fig. 39). The P.D. between the plane of shear and the body of the liquid is less than between the solid surface and the same (Curve 1). Also adsorption of ions may reduce or even reverse the charge in the adhering film without necessarily transmitting any effect to the solid phase (Curve 2). Thus electrokinetic potential (ζ) may be influenced without change of phase boundary potential (ϵ).

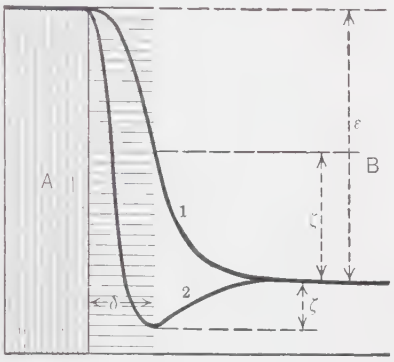

FIG. 39.—(Based on Freundlich.)

The relationship of electrokinetic potential to electrolytes will be considered more fully in dealing with colloids. Briefly it may be stated here that the discharging effect increases greatly with the valency (of the ion of opposite charge to the wall) and also with its adsorbability (see under Adsorption of ions).

4. Membrane Potential.—We have seen that a cell is not a single phase like an oil drop but that a watery phase forms the basis of the interior and an organophile phase predominates toward the surface forming an enveloping membrane. The question which most directly concerns us, therefore, is the condition necessary to produce a P.D. across a membrane. Regarding the membrane as a separate phase the condition obviously is that the phase boundary potentials at its inner and outer surfaces must not cancel out. For this relation to be satisfied (unless the

membrane itself have an organized polarity) the electrolytes must be different in the respective watery phases. If the membrane is permeable to the electrolytes only a temporary diffusion potential will be set up since ultimately the distribution on both sides will become uniform.

For a membrane potential to exist at equilibrium of diffusion it is necessary that at least one kind of ion shall be quite unable to pass through. This state of affairs results in an unequal distribution of ions known as the **Donnan equilibrium** and a membrane potential is sometimes called the **Donnan potential.** The result for the most part is the same whether the penetrating ions pass by pores or by solution in the membrane.

In a dynamic system like the living cell it is doubtful if we can assume that equilibrium of diffusion is ever reached, so that it does not follow because there is always a P.D. between the interior and exterior that the above condition as to permeability holds absolutely. Yet the fact that for certain cells at least the Donnan equilibrium has been found to be satisfied quantitatively requires that we devote some attention to it.

Take the simplest case of two electrolytes with a common cation divided by a membrane impermeable to one of the anions. At equilibrium the two diffusible ions will be distributed between the solutions, the impermeable anion will remain on its own side.

(1)		(2)
Na_1^+	|	Na_2^+
R^-	|	
Cl_1^-	|	Cl_2^-

Let these symbols represent the concentration of the ions indicated on the respective sides (1) and (2) of a membrane. R stands for the indiffusible ion.

Diffusion pressure tends to make $Na_1 = Na_2$ and $Cl_1 = Cl_2$. But the attraction of R^- makes $Na_1 > Na_2$ and its repulsion makes $Cl_1 < Cl_2$. In fact the electrostatic force so far outweighs the osmotic that, as far as chemical analysis can determine, cations = anions on each side, that is, there is electroneutrality. Actually the indiffusibility of R^- produces an almost infinitesimal excess of anions on side (1), and of cations on side (2), and it is this that produces the membrane potential. The value of this potential is given by a formula analogous to Nernst's formula for metallic

electrodes. At equilibrium, chlorine ions tend to be expelled from (1) with a force equal to $\frac{RT}{F} ln \frac{Cl_2}{Cl_1}$ which is opposed by their osmotic pressure, and Na ions are being attracted into (1) with a force $\frac{RT}{F} ln \frac{Na_1}{Na_2}$, also balanced by their osmotic pressure. But this is one and the same force, namely, the electromotive force across the membrane. Since, therefore, the two expressions are equal, $\frac{Na_1}{Na_2} = \frac{Cl_2}{Cl_1}$ or $Na_1 \times Cl_1 = Na_2 \times Cl_2 = Na_2^2$ (practically). Thus, knowing the relation of concentration of any two diffusible ions, the P.D. can be calculated. If another electrolyte with diffusible ions be present the same ratio will hold for each of its ions (if monovalent).

The presence of the indiffusible ion on side (1) also causes the final osmotic pressure to be higher on that side. The greater the proportion of diffusible salt the more is the effect of the indiffusible ion swamped both as regards its regulation of an osmotic difference and a potential difference across the membrane.

To sum up, we have seen that there are three possible causes of an E.M.F. due to separation of ions in physical systems analogous to that of the cell, namely, the slight and transient *diffusion potential* and the stable *phase boundary* and *membrane potentials*, which may amount to 0.01 to 0.1 or more volts.

5. Oxidation-Reduction Potential.—To complete the list of possible causes of bioelectric potential it is necessary to mention potential difference which is set up by a difference in oxidizing or, reducing power. Two solutions when connected through electrodes of the same noble metal (silver or platinum) may show a P.D. which is not explicable in terms of difference in concentration of a common ion but of difference in oxidation-reduction potential, that is, in their tendency to take up or give out *electrons*. Obviously, there are no metal conductors in cells, but some argue that the plasma membrane may conduct electrons, at least when stimulated.

II. CHANGES PRODUCED BY PASSAGE OF A CURRENT

1. Polarization.—The potential differences we have considered are those obtaining at equilibrium when no current is passing.

If a current is allowed to pass by connecting up the two ends of the chain (for example, the two solutions separated by a membrane), or if a current is sent through the chain from an external source, a redistribution of electrolyte is produced and along with it a change in E.M.F. which is called polarization.

If the fraction of the electric current transported by the cations (as compared with the anions) is greater in the membrane than in the solution, and correspondingly the fraction transported by the anions is greater in the solution than in the membrane, the concentration of electrolyte tends to be reduced in the immediate vicinity of the membrane on its + side, because the cations are accelerated on entering the membrane and anions on leaving it; and similarly the concentration is increased on the − side where retardation banks up the ions. Thus a membrane potential is set up and the original potential difference between the two solutions is altered. Also the solution becomes alkaline near the membrane on the + side and acid on the − side (due to the more rapid passage of H- and OH- than of other ions).

2. Electrosmosis and Anomalous Osmosis.—Electrosmosis under an applied current has already been discussed. It is possible that the same thing may happen as a result of currents derived from the energy of diffusion, as follows: With a membrane which is not perfectly semipermeable separating two electrolyte solutions, especially if dilute, osmosis does not follow the normal course as based on consideration of osmotic pressure alone. Depending on the electrolyte a solution may appear to attract water to a greater or less degree than it ought, or even to repel it. The latter condition, known as **negative osmosis,** is illustrated, for example, by a solution of tartaric acid or citric acid (say $M/3$) separated by pig's bladder from plain water. For a time water passes from the acid to the watery side. Acid, however, passes at the same time and, therefore, the solution does not become more concentrated—which of course would be contrary to the laws of energy. The explanation appears to be that the membrane is polarized, resulting in a flow of electric current which produces

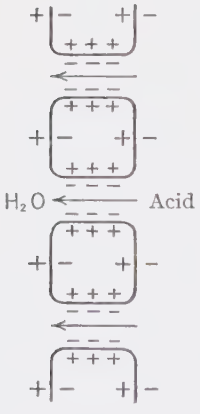

FIG. 40.

electrosmosis through its pores. In this particular case the membrane (protein) is negative towards water—or dilute acid—on the one side, while it assumes the positive sign in the stronger acid with a H-ion concentration beyond the isoelectric point of the membrane. Thus the two liquid media, taking on the opposite charge to the contiguous membrane surface, have a potential difference between them, the acid solution being negative. Another theory lays stress on diffusion potential in the pores as the cause of the P. D. The H-ions diffusing the faster leave the solution negative.

Lining the pore of the membrane we have a double layer, the solid wall of acid protein being positive, the movable layer negative. The movable layer, anions and water, migrates toward the positive (aqueous) side. The completion of the electric circuit probably takes place in the membrane itself, mainly through a passage of H-ions in the same direction as the anions are passing through the pores.

Although the exact mechanism of abnormal osmosis is not certain, experiment shows that it depends upon the ability of the ions to influence electrokinetic potential in a way which agrees with the above theory.

CHAPTER XII

ELECTRIC POTENTIAL AND ELECTRIC CURRENTS IN CELLS

In this chapter we shall only deal with that type of electric potential which expresses itself as an electromotive force and produces an electric current. Electrokinetic potential will be discussed more conveniently under Colloids.

1. Manifestations of Bioelectric Potential.—Just as the biologists Pfeffer and de Vries laid the foundation of our knowledge of osmotic pressure, so the physiological experiments of Galvani and du Bois-Raymond initiated the study of electromotive force. The former not only discovered that an electric current flows when a freshly cut nerve of a frog's leg touches the muscle—as shown by the twitch which results—but he also discovered unwittingly, as Volta pointed out, that contact of unlike metals produces the same result. Briefly, the parts of living bodies between which exist potential differences capable of producing a measureable current, are as follows:

1. *Related organs and tissues,* as muscle v. tendon, leaf v. stem, or the various tissues in the cross-section of a plant stem. (The cells with which the electrodes are in contact are here assumed to be uninjured.)
2. *Freshly cut or injured v. uninjured tissues* of one and the same organ. The injured part is negative by 1 to 10 millivolts. This is called the **current of injury.**
3. *Excited v. unexcited regions of one organ.* **Current of action** or **demarcation current.**—The excited area is negative. Similarly the potential difference between an uninjured and an injured part is reduced as the wave of excitation sweeps over the former—the so-called **negative variation.**
4. *Different parts of the same organ or cell in contact with different electrolytes or different concentrations of the same electrolyte.* **Current of rest.**

5. *Interior v. exterior of a single cell.*—The interior of protoplasm is negative to the exterior. The potential difference as measured by microelectrodes is of the same order as between injured and uninjured tissue. The vacuole of a plant cell, however, may be positive to the exterior.
6. *Between points along an internal stream of cytoplasm.*—The potential is slight—only a few millivolts—and disappears with cessation of movement (Gelfan).

Ordinarily a bioelectric potential amounts only to a few hundredths of a volt but when a large number of electromotive structures are arranged in series, as in the electric organs of certain fishes, a current of hundreds of volts may be produced—one of 450 volts has been measured in the electric catfish (*Malapterurus*).

The high hopes which were at first entertained when the fact of the production of electricity by living cells was discovered—hopes even of a solution of the problem of life in terms of electricity—were doomed to disappointment. Yet the measurement of electrical changes is being more and more used in physiology as an indication of other changes. Though the electrical may be only a small part of the transformations of energy which takes place in cells, they are often the easiest to observe and to measure. In the transmission of an impulse in a nerve, they are practically the only change that can be detected. Also, it is possible to make a continuous and graphic record of variations of potential, which allows the time relation of physiological processes to be followed.

2. Causes of Electric Potential in Cells.—To interpret these electrical changes, however, it is necessary to know something of their cause. Perhaps the only type of cell of which the properties have been determined with sufficient precision to allow a reasonably certain inference as to the origin of a potential difference is the mammalian red-blood cell. It is probable that conditions are simpler in so automatic a mechanism as the enucleated erythrocyte as compared with more vitally active cells. But the condition which exists in the former affords a suggestion for the study of other cells. Before dealing with this condition let us consider briefly how far the various conditions which have been enumerated in the previous chapter as giving rise to a potential difference may apply to living cells and tissues.

(*a*) *Diffusion Potential* no doubt exists but bioelectric poten-

tials are too large and too stable to be explained merely in terms of unequal speed of migration of ions.

(*b*) *Phase Boundary Potential* has many advocates since Beutner and others have shown that by this mechanism potential differences of the values observed in tissues can be produced and that many of the effects of ions on oil-water potentials are analogous to those at cell boundaries. In view of the evidence, which has been recorded elsewhere, that protoplasm is not a homogeneous "oily" phase, but consists of an internal aqueous phase enveloped by a water-immiscible one, it follows that the potential difference between the interior and exterior of a cell, if it is a phase boundary phenomenon at all, must be the resultant of two phase boundary potentials, one on each side of the plasma membrane, i.e. a membrane potential.

(*c*) *Membrane Potential.*—As a matter of fact we do not know whether ions pass through the plasma membrane by solution—which is the condition for phase boundary potentials—or in some other way, as by adsorption, chemical combination or passage through pores. Nor is it necessary to know the mechanism of their passage to predict the effect of ion concentration on the potential across the membrane. All that is necessary to give a transient potential difference is that one ion penetrate faster than the other; while to maintain a potential at equilibrium the condition is that certain ions of one sign should be able to pass and certain of the other sign should be unable—as will soon be shown to be the case. For practical purposes, therefore, we might regard the equilibrium potential difference in cells as a membrane (or Donnan) potential. With living membranes, however, a further complication appears. Even when the electrolyte solution is identical on both sides of a layer of protoplasm a P.D. may exist. When *Nitella* cells, for example, are bathed in their own sap the vacuole is + to the outside, and when living frog's skin has identical solutions on either side of it the inside is − to the outside. Evidently, there is a polarity in these membranes themselves. We have little knowledge at present as to how far cell potentials are due to such organized polarity of structure in the plasma membrane. There is good evidence, however, that separation of ions due to selective permeability is an important factor and one regarding which some quantitative data are available.

3. Evidence of Selective Permeability to Ions.—A Donnan potential of the type which has been the subject of physical experiments, namely that which is set up across a membrane impermeable only to colloids, cannot satisfy the conditions in living cells for several reasons:

(1) It is unable to give a sufficiently large potential difference.

(2) It demands that osmotic pressure inside and outside be unequal, whereas cells in the animal body are usually isotonic with the serum.

(3) It fails to account for the actual distribution of many crystalloidal ions.

On the other hand, impermeability of the plasma membrane to every ion could not give any potential difference at all. The question, therefore, is whether cells show a selective permeability to inorganic ions. The researches of Van Slyke and his co-workers have shown that blood cells at least are impermeable to crystalloidal cations and to more complex anions but permeable to simple anions such as Cl', Br', HCO_3'. Permeability is shown by exchange with other penetrating ions.

Whether $H^{\cdot}$ can penetrate or not is uncertain because the free passage of CO_2 should have much the same effect. By the mass law $\frac{[H^{\cdot}] \times [HCO_3']}{[H_2CO_3]}$ is constant. Assuming that the concentration of CO_2 and therefore of H_2CO_3 is approximately the same in the cells as in the plasma $[H^{\cdot}]$ must vary inversely as $[HCO_3']$ in both.

Since Cl' and HCO_3' are diffusible and $H^{\cdot}$ is at least virtually so, it follows if the equilibrium is of the Donnan type that

$$\frac{[H^{\cdot}]\ \text{inside}}{[H^{\cdot}]\ \text{outside}} = \frac{[Cl']\ \text{outside}}{[Cl']\ \text{inside}} = \frac{[HCO_3']\ \text{outside}}{[HCO_3']\ \text{inside}}$$

According to measurement

$$\frac{[H^{\cdot}]_i}{[H^{\cdot}]_o} = 0.77 \quad \frac{[Cl']_o}{[Cl']_i} = 0.62 \quad \frac{[HCO_3']_o}{[HCO_3']_i}$$

The agreement is not very close; but $[H^{\cdot}]$ inside can not be measured with accuracy, and other neglected factors render complete harmony improbable. Most important, however, is the fact that if one ratio is varied the others vary as would be expected if the theory holds true.

As regards other cells many mammalian tissues resemble blood cells in permitting an exchange of simple anions while at the same time their osmotic relations point to semipermeability to certain ions. The Cl content of the tissues is probably nearly all ionic since it is practically all in solution. Assuming that the bulk of the water can act as solvent, the ratio $\frac{[Cl'] \text{ inside}}{[Cl'] \text{ outside}}$ is known from analysis. $[H^+]$ outside is also known. Therefore, $[H^+]$ inside and the P.D. between the inside and outside can be calculated. The results for certain tissues and certain experimental results for comparison are shown in the following table from Haldane :

Tissue	Per Cent H_2O	Per Cent Cl	$\frac{a_i}{a_o}$	pH Calc.	pH Obs.	P. D. Calc.	P. D. Obs.
Red blood corpuscles	63.6	0.178	0.59	7.17	7.23	14.2	
Brain (white matter)	70.0	0.155	0.45	7.06	.....	20.7	17–28
Brain (gray matter)	81.5	0.115	0.30	6.87	.....	32.7	
Smooth muscle	80.0	0.112	0.29	6.87	.....	32.9	
Liver	84.0	0.096	0.24	6.72	6.40–7.04	38.3	
Striped muscle	76.0	0.061	0.168	6.62	6.02–6.91	47.8	40–80

$$\frac{a_i}{a_o} = \frac{[Cl'] + [HCO_3'] \text{ inside}}{[Cl'] + [HCO_3'] \text{ outside}}$$

It is difficult, however, to extend the principle of ready penetration of simple aions to cells in general. According to Hoagland and Davis the exchange of Cl′ and Br′—though it exists—in *Nitella* is an extremely slow process. A month may be required to reach equilibrium. Cl accumulates in the vacuole only under the action of light. Normally the ratio $\frac{[Cl'] \text{ inside}}{[Cl'] \text{ outside}} = \frac{100}{1}$, much too high to be explained as a Donnan equilibrium seeing that the vacuole is positive by only about 14.5 millivolts; and the $[H^+]$ instead of balancing the $[Cl']$ is also greater inside. Further investigation of ion exchange is necessary before it can be decided

whether a Donnan equilibrium and potential can exist in the majority of cells.

It is obvious, also, that even where a membrane potential has been shown to exist, the problem of how the unequal distribution of the ions to which the membrane is impermeable originally came about, or how it is restored after a change of permeability has allowed them to pass (see later), remains untouched.

4. Interpretation of Bioelectric Currents.—The *membrane potential* theory, however, is useful inasmuch as it correlates the various bioelectric phenomena. Thus the current of injury is explained as being due to increase of permeability, to passage of ions and to the approximation of the potential at the outer surface of the membrane at the point of injury to that at its inner surface. Similarly the current of action is what might be expected since a variety of independent evidence points to an increase of permeability with excitation of cells in general. The membrane theory of potential is an old one but in its original form as expressed by Bernstein it regarded superior penetration of cations as responsible.

This view was based largely on the **concentration effect.** The surface of a cell or tissue bathed by a concentrated solution is negative to one bathed by a more dilute solution. Were there no effect on the membrane itself we should have to conclude that the cations penetrate faster than the ions. It may be so. But solutions of single salts usually increase permeability, the more so the more concentrated they are, and thus another possible explanation of the negativity is the reduction of the polarity of the membrane by its general increase of permeability. The fact that calcium salts and in a less degree those of other divalent cations, are positive to salts of the alkalis when both are applied to the same cell or tissue is quite in harmony with the respective effect of these ions on permeability. It is, also, highly significant that organic cations which are particularly soluble in lipoids and electromotively active in oil chains, have no particular tendency to negativity when applied to cells.

The general agreement between the order of ionic effect in producing negativity and in augmenting excitability (Hoeber) also points to a change in the membrane itself. As regards anions, however, the order of negativity may well be the reciprocal of that of penetrability. It follows the lyotropic series $SO_4'' > Cl' > I' > SCN'$.

Recently the potential relationships of single cells have been extensively investigated by means of microelectrodes—usually a micropipette filled with agar saturated with KCl and containing a silver wire coated with AgCl, in other words, an electrode reversible to Cl-ions. It is unlikely, in view of the tendency to form new membranes, that the agar is really in contact with the internal protoplasm. The results for what they are worth are in harmony with the view that the interior of protoplasm is normally negative to the exterior. Vacuoles, however, may be either + or − to the exterior. The P.D. varies with the nature of the external medium.

We have considered so far only P.D. between the interior and exterior of cells but potentials also exist between different parts of tissues and resulting continuous currents have been recorded in certain plants and animals. The problem of how this E.M.F. is maintained is similar to the problem of how a cell which has been stimulated and more or less discharged regains its original state. In other words, how is the mobilization of ions effected. Lund [1] finds that tissue potential is correlated with oxidative activity of the cells. We may, therefore, consider briefly how this may apply to individual cells.

5. Oxidation-Reduction Potential Difference.—When one endeavours to measure potential difference between the interior and exterior of cells with platinum electrodes, the results probably depend mainly on the relative oxidation-reduction potential of the two regions. Dyes which, in contact with the atmosphere, remain oxidized are reduced in cells. This difference is not simply dependent on difference in oxygen pressure but depends on the presence of some reducing substances in the cell. Since reduction potential is equivalent to electron-giving potential, it tends to create an excess of negatively charged ions within the cell, giving rise to the type of negativity which is measured by differences in ion concentration. To maintain this potential difference when a current flows, the supply of reducing substance must be kept up by oxidation. It is a sign of the complexity of organization in the cell that, while dyes, etc., are reduced there which are not reduced in air, foodstuffs are at the same time oxidized which at ordinary temperatures are not oxidized in air. Possibly the location is different for the two operations. At any rate it follows on this theory, that oxidation may be the direct source of bioelectrical

[1] Lund, E. J. Am. J. Bot. *16:* 846 and 858 (1929).

energy. Indirectly, of course, it supplies energy for nearly all cell processes, except photosynthesis.

A point in favour of the oxidation-reduction theory of cell potential is the rather remarkable analogy which a certain physical model supplies. R. S. Lillie (see "General Cytology") has demonstrated that the passage of an electrochemical change along a "passive" iron wire resembles in many ways the transmission of a stimulus by nerve and other excitable tissues. In the physical model the events depend upon external oxidation and reduction of the wire so that a change of potential difference takes place between its surface and exterior. It has been suggested that similar changes in surface oxidation in nerve, etc., may account both for the passage of the stimulus and for the accompanying currents. Lillie's iron wire is "passive" as long as it lies undisturbed in the bath of HNO_3 which has oxidized its surface. But if the surface is scraped at any point a reaction accompanied by effervescence and disintegration of the oxide film sweeps along the wire at a pace comparable with, though much slower than, transmission by nerve. (Fig. 41*a*.)

The removal of the oxide film allows further oxidation to take place, making the part thus "activated" negative to the "passive" region of the wire. A current of positive electricity thus flows through the wire from the passive to the active region, escapes from sides of the wire to the solution and returns via the solution to the passive part. In the wire the current is electronic, causing cathodic reduction of the oxide film so that near the starting point where the reduction is strongest the film is destroyed and a new active area develops. Thus the circuit continuously repeats itself along the wire. The reducing effectiveness of the current decreases in the order A > B > C. Beyond a distance *XY* it is unable to activate.

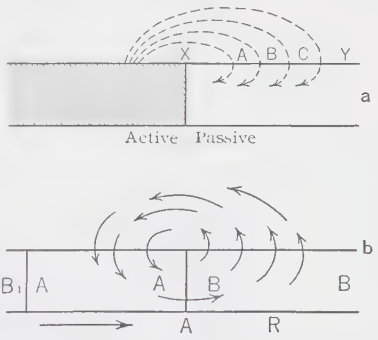

Fig. 41.

Adapted by permission from R. S. Lillie in E. V. Cowdry's "General Cytology," published by the University of Chicago Press.

This model has many resemblances to transmission in pro-

toplasmic systems. Among its advantages it exhibits an automatic restoration of the oxide film and of the original state in general, after the wave of activity has passed. An essential feature of the theory as applied to protoplasm would seem to be that the membrane when excited shall conduct electrons instead of merely allowing ions to pass. Whether such a conception is allowable is, however, questionable (see Mathews in General Cytology). But, whether by means of oxidations or not, transmission of electric potential undoubtedly accompanies transmission of any stimulus.

In the second diagram (Fig. 41*b*) the area *AA* represents the region of the nerve-axone which is active, or occupied by the excitation-wave, at the instant under consideration; its length in a frog's motor nerve at 20° is about 6 cm. The wave is regarded as moving in the direction of the large arrow. The region now undergoing secondary electrical stimulation by the local action-current flowing between regions *AA* and *BB* extends to the distance *AR* (ca. 3 cm.) beyond the wave-front. The direction of the current (positive stream) in part of the local circuit is indicated by small arrows. The region (B_1) immediately behind the excitation-wave is temporarily in a refractory state.

Transmission in nerve is to be regarded not as a unique or specialized function peculiar to this and similar tissues, but as an example of a type of process occurring everywhere in irritable protoplasm. The special features of the process (its velocity constants, metabolic and other features, etc.) vary widely in different forms of protoplasm; but the general underlying chemical and structural conditions are apparently of the same fundamental nature in all cases. The fundamental condition is the presence of thin polarizable partitions or films, consisting largely of chemically alterable material, and separating chemically dissimilar regions which are at the same time electrical conductors.

—Lillie.

6. Functional Significance of Bioelectric Potential.—Except in few specialized cases where powerful currents are produced which apparently have a protective function, it is uncertain whether bioelectric potential and the currents which invariably accompany stimulation serve any useful purpose in themselves or are merely incidental. Various speculations as to their possible rôle have been put forward.

It is suggested, for example, in the absence of special conducting tissue and without protoplasmic connection between cells—since

these are not frequently demonstrable and since stimuli can pass across a gelatin layer electrochemical processes in one cell may influence those in another by passage of a current through the wall. Thus correlation of growth and development may be brought about. A close relation between the structural symmetry of a plant and its electrical polarities is claimed (Lund).

It is further suggested that *secretion and absorption* may be regulated by electrical polarity of membranes. Water and ions often pass through gut and glandular epithelium in animals and the epidermis of roots contrary to osmotic pressure. We have seen that water may do so electrosmotically across a membrane which is polarized and permeable to ions. If an epithelial layer is polarized the same thing may happen during periods of activity when permeability is heightened—a current may circulate, for example, through the cells and back through the intercellular spaces. In this connection it is important to note that the extreme thinness of the plasma membrane causes the gradient of potential to be proportionately steep, so that a given P.D. between the interior and exterior of say a red blood cell may create a field 30,000 times the intensity of that existing in an ordinary collodion membrane having the same P.D. between its opposite sides. This may account for the relative velocity of absorption and excretion by cells in some cases.

7. Effects of Passing an Electric Current through Tissues.—Although the rôle of bioelectric currents in vital processes is only speculative, the effects of passing an external current through tissues are better known.

(*a*) *Polarization and Changes in Ion Concentration.*—This can be visibly demonstrated as shown by Bethe. When a continuous current is passed through cells of the stem of species of *Tradescantia*, which contain a violet indicator pigment in their sap, the ends of the cells toward the anode become green (alkaline) and towards the cathode red (acid) just as with a physical membrane, as of gelatin. With a 10-volt current, half a minute is sufficient for the polarization to appear. While this polarization is developing, the resistance of the tissue rises. The true resistance of the cell interior is got by using a rapidly alternating current, though here again the passage of current is impeded by the charging up of the membranes as condensers. Capacity has to be considered (see p. 89).

(*b*) *Stimulation.*—The most fruitful application of the phenomenon of polarization to explain the effects of an electric current is seen in Nernst's **theory of stimulation.** The theory is that stimulation takes place when, through polarization, a certain definite increase—or decrease—of ion concentration takes place at some particular membrane. It is shown mathematically that no matter what kind of current is used the same concentration of ions is probably attained at the threshold of stimulation. Presumably the colloidal effect on the plasma membrane of the change in concentration is responsible for the increase in permeability that accompanies excitation.

That the passage of an electric current does increase permeability can be directly proved by a simple experiment. If *Spirogyra* lying in a solution of an acidic dye such as acid fuchsin or cyanol, to which it is normally impermeable, is subjected to a suitable alternating current or a continuous current reversed every 5 or 10 seconds, the cell sap quickly becomes coloured.

What the real order of cause and effect in transmission of a normal nerve or protoplasmic impulse is—whether an initial current causes increased permeability or whether increased permeability causes a current (by allowing ions to pass)—is not clear.

Altogether, bioelectric phenomena, though useful as an indication of activity, throw a very uncertain light as yet on the mechanism of that activity.

The Importance of Colloids in Vital Phenomena

More perhaps of the characteristics of the living substance are explicable on the basis of its colloidal nature than on any other ground. The properties of the colloidal state, embracing as they do all that has gone before—properties of surfaces, properties of solutions and electrical phenomena, are a fitting climax to our study. Our interest is not confined to the behaviour of colloids in the mass, but to the potentialities of the elemental particle that constitutes the colloidal state; because the first faint signs of life are displayed by bodies of that order of size and complexity.

CHAPTER XIII

COLLOIDS IN PHYSICAL SYSTEMS

I. GENERAL

1. General Characteristics of the Colloidal State.—Thomas Graham, in the middle of last century, discovered that solutions of certain substances such as glue, gelatin or starch, differ markedly from ordinary solutions in several respects. The rate of diffusion of these substances he found to be relatively slow and the osmotic pressure of their solutions insignificant, both indicating that the state of dispersion is not so fine as in a typical solution. The kinetically active particles must be fewer and larger. The particles are not so large as to be held back by any kind of filter paper, nor are they visible under the highest powers of the ordinary microscope. They are unable, however, as Graham showed, to pass through parchment; their solutions are opalescent in a strong beam of light; amd, as a subsequently developed technique has demonstrated, the individual particles may be perceived with the aid of the so-called ultramicroscope. The process of separating colloids from substances in true solution (crystalloids) by filtration through parchment and similar membranes Graham termed *dialysis*. He gave the name **colloids** to non-dialysable substances from the Greek word **kolle** (κολλη) glue. He regarded them as a class of substances which are amorphous in the solid state and form solutions with the properties indicated above, whereas crystalloids are crystalline and form true solutions.

It has since been shown that the ability to exist in the colloidal state of dispersion is not confined to any class of material. Graham investigated only those that naturally go into solution in this form, but, either by precipitation from a finer state or by mechanical or electrical disintegration from a more massive state, all kinds of substance, since Graham's day, have been transformed into colloids. We, therefore, speak now not of colloidal substances but of the colloidal state of matter.

The peculiar behaviours of substances in the colloidal state, which make it necessary to study them independently, depend upon the fact that, though possessing in a certain degree the properties of solutions, they also exhibit in the highest degree those of surfaces. The finer the state of subdivision of matter the higher the ratio of surface to volume:

$$\frac{\text{Surface}}{\text{volume}} = \frac{r^2}{r^3} = \frac{1}{r}$$

The smaller the r, the larger is the fraction. The adsorbing capacity of a given quantity of dispersed substance, therefore, increases in proportion as the radius of its particles decreases. There is a limit, however. At molecular size adsorption ceases. Whether (minimum) size or (maximum) motion is the limiting factor is uncertain. In that intermediate state of matter known as colloidal, capillary phenomena obtain their greatest expression. To this, in fact, is due more of the characteristic properties of colloids than to kinetic activity, because, although the latter is manifest only in colloidal solutions or *sols*, jellies or *gels* are so closely related that they must necessarily be considered in the same connection.

2. Optical Properties of Colloids. (*a*) *Tyndall Effect.*—Colloidal solutions may appear perfectly clear under ordinary conditions of lighting, but, if observed against a dark background under the illumination of a transverse beam, the illuminated band presents a milky or opalescent appearance. The conditions are the same as when motes in the atmosphere are rendered visible by a beam of light entering a darkened room, namely lateral illumination and a dark field or background. The analogy is not complete, however. The light which comes from the dust particles is reflected light, whereas from the much smaller colloidal particles it is diffracted light. A particle does not reflect radiations of wave length more than twice its own diameter. The wave lengths of visible light range 0.4 to 0.7μ; consequently, the smallest particle which reflect or refract visible light must have a diameter of at least 0.2μ. Truly colloidal particles are usually smaller than this. Below this limit particles still deflect light by diffraction—provided, of course, that their index of refraction differs from that of the medium they are suspended in. Diffracted light is scattered in all directions so that the amount sent out in any one

direction is very small and can be seen only against a dark background. A peculiarity of this light is that at right angles to the incident beam it is entirely polarized. This whole phenomenon is known as the **Tyndall effect.**

(*b*) *Appearance under Ultramicroscope.*—The different forms of ultramicroscope render the individual particles visible in the same way as the Tyndall effect reveals them *en masse.*

The Zsigmondy or slit ultramicroscope makes use of the illumination of an extremely thin layer of solution. This is procured by focusing a lighted slit horizontally within it. The microscope is set at right angles to the beam, the objective being immersed in the solution (Fig. 42). For biological purposes the most convenient type of ultramicroscope is that in which oblique

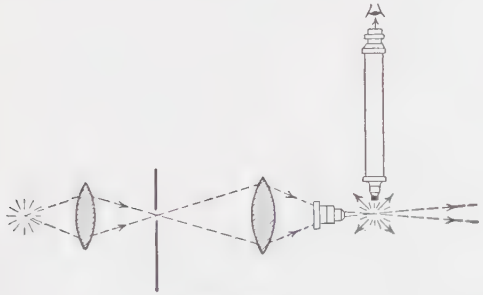

Fig. 42.

lateral illumination from all sides is obtained by the aid of a dark field condenser (Fig. 43). Such a condenser is medianly opaque and admits only a narrow ring of light which is so reflected as to converge in a very wide-angled hollow cone. The apex of the cone should be at the level of the liquid or object to be examined. The angle must be sufficiently wide to prevent the direct beam from entering the objective; that is, the lens when focused must lie within the dark center of the upper (inverted) light cone. With the highest power it is generally necessary to stop down the aperture of the objective.

When the upper surface of the coverglass is in contact with air, the light is largely reflected from it. This enables one to illuminate an object from above by simply raising the condenser and with it the apex of the light cone until its reflection falls on the desired spot. (Fig. 43*c*.) This technique is especially

advantageous in detecting fluorescence, as thereby the dazzling effect of too much refracted and reflected light is eliminated.

The visibility of colloidal particles in a dark field, as already pointed out, depends mainly or entirely on the scattering or diffraction of light. Diffraction does not produce a true image of the particle. Neither its shape, size, nor colour is directly indicated by the shape, size and colour of the image. What is seen is not the particle but the base of a cone of light of which it is the apex. The center of the image so perceived is a small bright disk and the periphery a series of rings widening outward, the usual " inter-

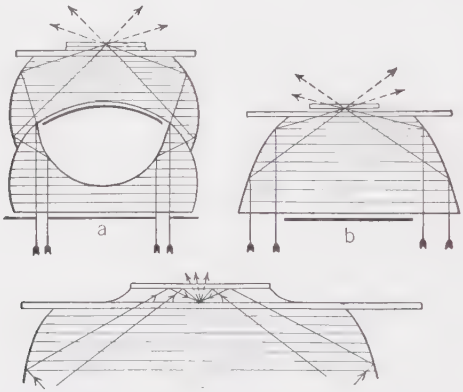

Fig. 43.—Diagram of types of dark field condenser. (*a*) Cardioid type, (*b*) parabaloid type, (*c*) reflection from coverglass when in contact with air.

ference " effect. A much elongated particle may be recognized by its oval rings and a flat one by its twinkling appearance; otherwise shape is indeterminable. The colour is the same as in the macroscopic Tyndall effect and depends in a rather complex way on the size of the particle. Generally the shorter wave lengths—blue light—are scattered most.

Although the size of an ultramicroscopic particle cannot be estimated from its appearance, the average size can be calculated from the number of particles in a given volume. Zsigmondy found that gold particles were visible down to $6\mu\mu$ (millimicrons) diameter, but there is no definite size limit of visibility, because the more intense the illumination the lower the limit. Because the amount of light diffracted depends on the refractive index as well

as the size of the particles, most substances become invisible at a much higher limit of size than in the case of metals. In fact a large group of colloidal substances, and the most important in biology, form solutions of which the particles are normally invisible under the ultramicroscope. This is partly due to small size and partly to the small difference between the refractive index of the medium and of the particle —which is itself hydrated. The visible class is named **suspensoid** and the invisible commonly **emulsoid.** Other distinctions between these two classes are mentioned below.

(*c*) The **Brownian movement** of particles is a very striking phenomenon when viewed with the ultramicroscope, though it is still visible in particles of microscopic size. The path of the particles can be plotted out roughly by a camera lucida or by a moving picture camera, but as their direction of movement changes with enormous frequency—probably millions of times per second—their exact path cannot be plotted nor their speed determined directly. Thanks, however, to a formula[1] derived by Einstein relating the radius of particles with the (measureable) *mean displacement* in a given time, Perrin was able to show that particles obey the same laws as molecules. Avogadro's constant is the same, independent of size, and $\frac{1}{2}\ mv^2$ for molecules equals $\frac{1}{2}\ mv^2$ for particles.

Brownian movement is somewhat oscillatory, especially with larger particles, and the amplitude of the oscillations gives one a rough idea of the magnitude of the mean displacement. It varies inversely as the square root of the radius of the particle and as the square root of the viscosity of the medium. Judging by particles of similar size we are thus able to estimate relative viscosity.

3. Grades of Dispersion.—The continuous gradation of the changes in properties as particles become finer and finer and the subservience to the same laws throughout are reasons for saying that substances in the colloidal state exhibit properties of solutions. Their resemblance to solutions, however, as well as other of their characteristics vary notably with the size of particles or the degree of dispersion, as it is called. The accompanying table shows the size limits at which colloidal properties begin and end.

[1] The formula for the mean displacement is $\sqrt{\frac{RTt}{N3\pi\eta r}}$ where t = time, r = radius of particle η = viscosity of medium, R = gas constant, T = absolute temperature and N = Avogadro's constant, the number of active particles in 22.4 liters when the pressure = 1 atm.

Size Limits of Colloidal Properties

2μ	$500\mu\mu$	$200\mu\mu$	$5\mu\mu$	$0.1\mu\mu$
Microns (Visible with Ordinary Microscope)		Ultramicrons (Visible with Ultra-microscope)		Amicrons (Invisible)
Suspensions		Suspensoids		Molecular dispersion (true solutions)
Filterable		Dialysable		Non-dialysable except by semi-permeable membranes.
Unstable (settle out on Standing)		Relatively stable		Stable

One sees that the colloidal state may embrace particles ranging from about 500 to 5 millimicrons diameter. As there is a gradation downward to molecules so is there one upward into coarse dispersions—called suspensions if the particles are solid, emulsions if they are liquid. Whether coarse or colloidal and whether solid, liquid or gas, the separate "particles" are termed the **disperse phase** or **internal phase**, whereas the medium they are dispersed in is called the **dispersion medium** or **external phase.**

The breaking up or separating of particles into finer ones is called **dispersion,** their coming together **aggregation.** Dispersion from a large mass is **peptization,** aggregation into a mass is variously termed **flocculation** (forming floccules), **precipitation** (throwing down), **coagulation** (forming a clot), or **gelation** (setting to a jelly), all with distinct shades of meaning.

Reversal of Phases.—When both phases are liquid an increase in the proportion of internal phase or a change in surface tension relations may bring about a reversal of phases, as is illustrated by experiments with emulsions. When equal parts of olive oil and water are shaken up with NaOH the oil forms the internal phase; with $CaCl_2$ the water is internal. Probably the side of the interface with lower tension tends to be external, having less tendency to reduce surface. The Na soap, which is formed from NaOH and oleic acid, dissolves in water and lowers its surface tension, the Ca soap is more soluble in oil.

4. Suspensoids v. Emulsoids.—It was noted above that although all colloids may show the Tyndall effect, a large propor-

tion of them fail to show up as discrete particles under the ultramicroscope. There may be diffuse illumination or the liquid may appear optically empty. This optical distinction is attended by other differences which mark out two classes of colloid. The names commonly applied to these respective groups are **suspensoid** and **emulsoid.** These terms unfortunately suggest that we are dealing with the colloidal equivalents of *suspensions* and *emulsions*, that is, with dispersions of solid and liquid particles respectively. But emulsoids are not fine emulsions, in the sense that their disperse phase is a liquid immiscible with the dispersion medium. Their substance if dried out is usually a solid; *the essential feature is that it freely absorbs the dispersion medium.*

The names **lyophobic** and **lyophilic,** or, if the medium is water, **hydrophobic** and **hydrophilic** (solvent hating and solvent loving, or water hating and water loving) are more appropriate than any other, because they express the fundamental difference on which other distinctions depend. In brief, these distinctions are as given in the table herewith.

Distinctions between Hydrophobic or Suspensoid and Hydrophilic or Emulsoid Colloids

Property	Suspensoids	Emulsoids
Visibility	Ultramicroscopically visible	Invisible
Viscosity	Practically equal to that of water.	Much higher than water.
Surface tension	Practically equal to that of water.	Usually low.
Stability	Depends largely on electric charge; hence precipitated by dilute electrolytes.	Depends mainly on affinity for dispersion medium (partial solubility); hence precipitated only by high (desolvating) concentration of salts, etc.
Peptizability (dispersability)	Difficult. Often irreversible after coagulation.	Disperse spontaneously or on heating; thus reversible.

Substances which form suspensoids in water are: metals, insoluble salts, fats and oils; those which form emulsoids are: proteins, carbohydrates and many other organic substances. As usual the two classes grade into one another. Metallic oxides, for example, show intermediate properties. So do many organic compounds.

II. SUSPENSOIDS

1. Factors Regulating Dispersion.—Brownian movement tends to bring particles in contact and surface tension to make them adhere. If an adsorption film of liquid prevents contact from becoming complete immediately, Brownian movement may again cause separation. But actually it is found that suspensoid particles remain dispersed only when they bear a certain electric charge. The electrostatic repulsion of like charges prevents contact.

The sign and potential of the charge on particles may be determined by *electrophoresis* (migration in an electric field). The migration velocity may be determined microscopically or ultramicroscopically by observation of individual particles. It may also be determined, though with less precision macroscopically, from the rate at which the boundary between a colloidal suspension and the pure solvents moves in either limb of a U-tube. In both cases it is assumed that a constant current of known gradient of potential is passing through the liquid. The velocity of a particle is proportional to the electrokinetic potential on its surface and is independent of its size and shape.

Hardy found that a colloidal solution remains stable as long as the particles possess a charge, either + or −, but precipitates when they have none. The charge can be varied by varying the concentration of acid or alkali. The H-ion concentration which produces electrical neutrality he called the **isoelectric point.** Other electrolytes also influence the charge, depending on the relative adsorbability of cation and anion as described under adsorption. It was later shown (Burton, Powis) that precipitation and flocculation take place before the null point is reached—in fact as soon as the electrokinetic potential is reduced to a certain critical value, which varies, however, with the character of the suspensoid and sometimes of the electrolyte.

Critical Potential (Millivolts)

Coalescence of oil drops	KCl	30	$BaCl_2$	28	$AlCl_3$	30
Flocculation of As_2S_3	KCl	40	$BaCl_2$	25	$AlCl_3$	30
	HCl	50				

For graphite the critical potential is lower, namely: 13–17 mv. Most colloidal particles in pure water have a potential of 60 to 90 millivolts.

2. Flocculation by Electrolytes.—The principal laws are:

Only the ion of opposite sign is effective (Hardy).

The flocculating power increases greatly with the valency of the oppositely charged ion (Shultze, Linder, Picton).

The flocculating power decreases somewhat with the valency and adsorbability of the ion of like charge.

All these laws apply also to the effect of ions on surface charge in general. Precipitation of colloids is only one manifestation. The accompanying tables of *flocculation values* (critical concentration in millimols causing flocculation in a given time, namely, two hours) of different electrolytes will illustrate these laws.

Tables of Flocculation Values

I. Negative sol (As_2S_3 1.85 grams per litre)

	Flocculation Value in Millimols
$\frac{K_2SO_4}{2}$	66
KCl	50
KNO_3	50
$BaCl_2$	0.70
$AlCl_3$	0.09
New Fuchsin (basic dye)	0.11

II. Positive sol (Al_2O_3 0.823 grams per liter)

$\frac{BaCl_2}{2}$	90
KCl	80
K_2SO_4	0.28
$K_4Fe(CN)_6$	0.08

The valency effect is seen to be very powerful in precipitation and slight in stabilization. The effect of adsorbability is particularly noticeable in the case of organic ions, but it also enters into that of polyvalent as well as H- and heavy metal ions. The valency effect is predictable from the adsorption curve. Suppose

that mono- di- and trivalent ions have the same isotherm (Fig. 44) and that the concentration of adsorbed monovalent ions necessary to bring the potential to the critical point is A. Half that amount of divalent ion and one-third of trivalent will have the same effect. Marking out these ordinates A, $A/2$ and $A/3$, on the adsorption axis, we find that the corresponding concentrations C_1, C_2, C_3, in solution have somewhat the ratio discovered by experiment. The explanation is due to Freundlich. The assumption here made that flocculation depends directly on the equivalent amount of ions adsorbed is supported by chemical analysis.

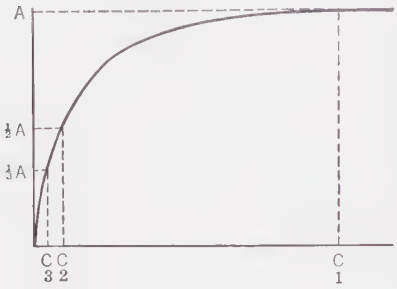

Fig. 44.

3. Relation of Electrokinetic Potential to Action Electrolytes.—Depending upon their concentration and nature, electrolytes not only flocculate but may also peptize suspensoid sols. A similar variable effect is evident in their action on the viscosity and osmotic pressure of emulsoids and on the swelling of gels. All of these rather complex relations may be correlated with the changes of electrokinetic potential which are induced by the various agents, and it will simplify our study of the colloidal phenomena to consider first this more fundamental one.

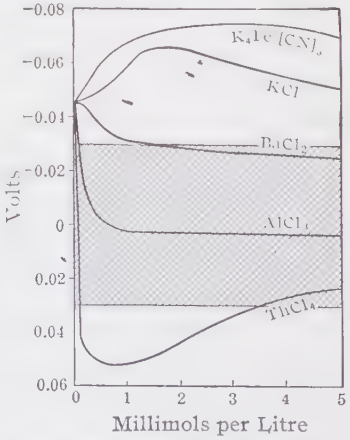

Fig. 45.—(After Freundlich.)

Figure 45 shows the relation between the potential (in volts) at an oil-water interface and the concentration (in millimols) of various electrolytes. Between +30 and −30 millivolts is the zone of flocculation for oil. The separation is more or less gradual, depending on the charge. At higher concentrations the potential with salts of the alkali cations also reaches the flocculating limit.

It is important to note that in low concentration these salts may increase the charge and, therefore, the dispersion of a negatively charged colloid, because it helps to explain why sometimes they oppose the action of polyvalent cations. Salts of a tetravalent cation rapidly decrease the charge to the flocculation point, then reverse the charge and re-peptize the colloid and in still higher concentration may again flocculate it (Fig. 46). With many colloids, trivalent ions behave in the same way. It is difficult to conceive of these variations as being entirely due to alternative adsorption of cations and anions respectively. A further explanation is probably to be sought in changes in the thickness of the double layer as explained on p. 133. The application of these results to the behaviour of emulsoids is given later.

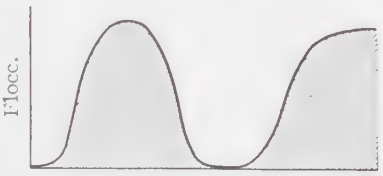

Fig. 46.—(After Kruyt.)

4. Periodic Precipitation—Liesegang Rings.—When one electrolyte diffuses into a solution of another with which it interacts to form a precipitate, the deposition of this precipitate is sometimes rhythmical, appearing as bands which gradually widen and separate outward as the gradient of diffusion falls. The phenomenon is rarely seen except in gels but the gel is only accessory as shown below, not essential. The exact mechanism is not known but various feasible hypotheses have been put forward. Take, for example, the case of $AgNO_3$ diffusing into $K_2Cr_2O_7$. As long as chromate ions are in excess the $Ag_2Cr_2O_7$ remains suspended either as molecules or as negatively charged colloidal particles. When a certain concentration ratio of silver to chromate ions is reached the charge of the particles falls below the limit of stability and flocculation ensues. In some way—either by adsorption or by serving as a nucleus for further condensation of $Ag_2Cr_2O_7$—the zone of precipitation draws chromate ions from that immediately beyond so that a space occurs in which little silver chromate can form. (A similar impoverization of silver salt around the margin of a dense precipitate may be seen in a photograph.) Beyond this more or less barren zone, the incidence of flocculation again follows the accumulation of sufficient Ag ions, and so the cycle is repeated. The rôle of the gelatin in periodical precipitation is partly to prevent

convection currents and partly to act as a protective colloid, delaying the crystallization of precipitate until the electrical mobilization has time to act. Thus a less protective gel, such as starch, may induce a diffuse precipitate or indefinite zones with the same concentrations of reagents as give clean-cut bands in gelatin (Lloyd).

III. EMULSOIDS

1. Fundamental Distinction.—The characters which distinguish emulsoids from suspensoids were summarized above. The fundamental distinction was seen to be their tendency to bind water—or whatever the solvent may be. Two characteristic features emerging from this basal property are: (1) their high viscosity; and (2) their stability in presence of electrolytes, that is, stability even when discharged. That both of these features depend on hydration (or solvation) of the particles can be proved very simply by dehydrating with alcohol. An agar sol, for example, which is typically emulsoid, immediately assumes on the addition of alcohol the properties of a suspensoid. Its viscosity drops practically to that of the medium (the alcohol-water mixture); it exhibits the opalescent Tyndall effect; its particles become visible under the ultramicroscope; and it is precipitated by dilute electrolytes. We see, therefore, that an emulsoid is simply a hydrated suspensoid.

2. Viscosity. (*a*) *Measurement.*—It is necessary to divert our attention for a moment to the meaning and measurement of viscosity in general. Differences in the viscosity of liquids are shown by differences in their rate of movement or flow, and measurements of viscosity may be made by measuring rates of flow. Viscosity depends on friction between the molecules of the liquid when one layer glides over another. Consider, for example, water flowing through a glass tube. The layer of molecules next the wall is practically stationary owing to the adhesion of water to glass. Owing to friction the next layer moves much more slowly than the body of the liquid and successive layers each a little less slowly. If the tube is a capillary one this shearing effect of friction extends to the center of the tube. If the tube is wide the liquid in the central region flows *en masse.*

The viscosity of a liquid may be defined as the force per unit area required to cause one layer of liquid to flow with unit velocity past another layer unit distance apart from it.

By using the same tube it is easy to compare the viscosity of different liquids:

$$\text{Viscosity} \propto \frac{\text{Force producing flow}}{\text{Rate of flow}}$$

If we use the same volume of liquid each time, the pressure is proportional to its density (d). The rate of flow varies inversely as the time (t) taken for a given volume to flow through the tube. Thus:

$$\eta_1 \propto d_1 t_1$$

$$\eta_2 \propto d_2 t_2$$

$$\frac{\eta_1}{\eta_2} \propto \frac{d_1 t_1}{d_2 t_2}$$

If one of the liquids is water its density is unity and its viscosity may also be taken as unity, or in absolute units 6.6×10^{-3} dynes per sq. cm. at 38° C.

Further practical details are given in Part II. Here we have to take up certain theoretical considerations which are important in the study of viscosity of emulsoids.

(*b*) *Phase Volume Effect.*—Einstein derived a theoretical formula for the effect on the viscosity of a liquid of adding a suspension to it, which says that the "relative increase of viscosity" varies as the relative volume of the internal phase:

$$\frac{\eta_s - \eta_0}{\eta_0} = 2.5\ \phi$$

where η_s is the viscosity (or internal friction) of the suspension, η_0 of the medium, and ϕ the volume of suspended substance in unit volume of suspension. According to the formula the viscosity is independent of the state of dispersion. If, however, a shell of adsorbed water surrounds each particle the volume of the disperse phase, including the water shells, will increase with subdivision. This applies particularly to emulsoids (which are strongly hydrated), and some ascribe their marked variations in viscosity to variations in degree of dispersion (Alexander).

(*c*) *Electroviscous Effect.*—Smoluchowsky deduced theoretically that if the particles are charged a function of the electrokinetic potential [$f(\zeta)$] must be introduced into the formula:

$$\frac{\eta_s - \eta_0}{\eta_0} = 2.5\ \phi\ [1 + f(\zeta)]$$

Apparently the charge has a much greater effect on emulsoids than on suspensoids because, although Einstein's formula holds approximately for suspensoids, the actual value of η_r in a dilute emulsoid may be hundreds of times greater than would be inferred from it. It is hardly likely that the particles are hydrated to such a degree as to increase their volume two or three hundred times. Probably the effects of hydration and electric charge combine. Kruyt and Jong have shown experimentally that the viscosity varies profoundly with the concentration of electrolyte—affecting the charge on the particles—as is shown in Fig. 47 for an agar sol. The experiments were carried out above 40°, at which temperature true viscosity unaffected by rigidity (see later) determines the results. This effect of electrolytes is termed the **electroviscous effect.**

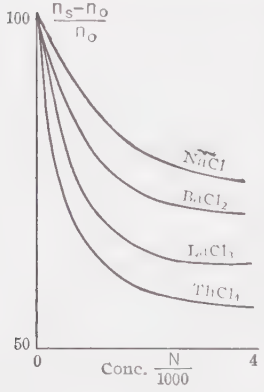

Fig. 47.—(After Kruyt.)

(*d*) *Viscosity of Proteins.*—The viscosity of protein sols in relation to electrolytes is similar to the electroviscous effect. Thus taking different concentrations of acid and alkali and plotting the *p*H of a gelatin sol against its viscosity we get a curve (Fig. 48) with a minimum at the isoelectric point and two maxima, one about *p*H 3, the other between *p*H 7 and *p*H 8. The electrokinetic potential varies in a similar fashion. The viscosity change is not entirely due to the direct effect of the charge but also to what may be an indirect effect, namely, that the hydration of the particles varies in a similar way with *p*H. This is indicated by the fact that the concentration of alcohol required to dehydrate and precipitate the gelatin is least at the isoelectric point and is also shown by the similarity of the hydration curve of gelatin gels.

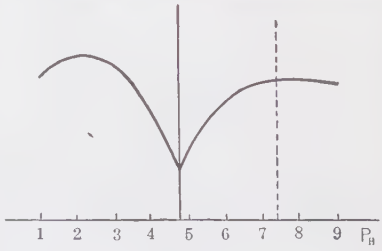

Fig. 48.

The effect of salts on the viscosity of gelatin differs with the

*p*H. If we start with isoelectric gelatin they increase viscosity in a low concentration and decrease it in higher concentrations, as might be expected from the effect of electrolytes on charge as described above (section 3). The maximum with trivalent ions appears at a very low concentration - about *M* 10,000, with bivalent at a higher, and with monovalent at a still higher concentration—*M* 2. The farther from the isoelectric point we start, that is, the greater the charge on the particles, the less tendency is there for added salts to increase the charge and viscosity; and a point is soon reached where even low concentrations of salt only depress viscosity.

The explanation here given of the effect of electrolytes on the viscosity of proteins is not really affected by the fact that acid and alkali probably unite with the gelatin micellae by chemical combination rather than adsorption of H- and OH-ions. The effect on charge is similar.

3. Rigidity of Emulsoid Sols—Sol Gel Transformation.—Emulsoid sols usually exhibit a certain measure of rigidity as well as viscosity. When a small paddle-wheel is set rotating in glycerine or concentrated sugar solution, it comes to rest asymptotically as ought to be the case when the damping is due to true viscosity, but in a sol such as albumen it stops only after a series of strongly damped oscillations. Similarly a particle of iron suspended in a sol and attracted by a magnet recoils when the displacing force is removed. It is evident from these experiments that rudiments of an elastic structure are present in emulsoids even in the sol state. At higher temperatures the elasticity disappears and at lower temperatures and higher concentrations of colloid it increases until the whole mass becomes an elastically rigid gel. In the case of the most typical gels rigidity is the only property in which they differ from the sols out of which they are formed. Such are gelatin, agar and soap gels. Gelation in such cases appears quite different from coagulation. But other gels grade into gelatinous precipitates which obey all the laws of precipitation. It follows from their elasticity that gels possess a cohering structure, involving a kind of aggregation of particles, but one in which the aggregation retains the shape of the sol. In those gels, such as blood fibrin or soap, where the ultramicroscope reveals a structure it is fibrillar, and many believe that this structure is universal, although in the most typical jellies it is too fine to be seen. The characteristic

properties of gels also depends upon the fact that the particles, though cohering, retain their hydration.

4. Swelling of Gels.—One of the most characteristic properties of elastic gels is that of swelling, that is, of reimbibing solvent after having been dried out. The degree of swelling of a gel mass depends on the electrolytes present, in almost the same way as does the viscosity of its sol, which as we have seen is a function of the charge and volume of the individual micellae. At the isoelectric point—*p*H 4.7 in the case of gelatin—swelling is at a minimum. Starting with isoelectric gelatin small amounts of any electrolyte increase swelling—large amounts decrease it. The relation to concentration and valency is shown diagrammatically in Fig. 49. Polyvalent cations tend to make the gelatin positive so that the isoelectric point is no longer *p*H 4.7 but somewhere on the alkaline side of this. Polyvalent anions make it negative and shift the isoelectric point in the acid direction. The effect of salt below a certain concentration depends almost entirely on valency and concentration. With higher concentrations—over *M*/4 the lyotropic series makes itself apparent.

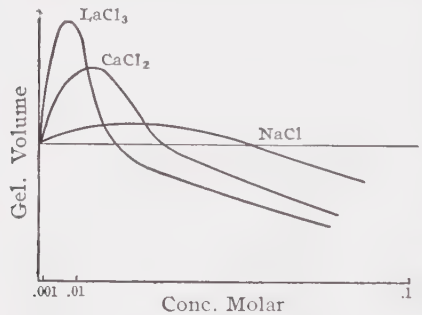

Fig. 49.

Order of swelling—Anions: $I > NO_3 > Br > Cl > H_2O >$ acetate > citrate > sulphate

The effect of acids and alkalis is the same as if H resembled a polyvalent cation and OH a polyvalent anion, although the effect is greater. But, as we have seen, with proteins, at least, the action of H and OH is chemical rather than one of mere physical adsorption. If the gel substance is already fully charged, either + by acid or − by alkali, the addition of salt can only reduce the charge and also the swelling. On the alkaline side of the isoelectric point, salts with polyvalent cations have this effect preeminently; on the acid side, salts with polyvalent anions. Thus a form of "antagonism" is exemplified. Figure 50 shows the relation

of the swelling of gelatin to *p*H and the effect of salts in concentration $M/100$ on acid and alkaline gels.

Swelling Pressure.—The relative effect of various electrolytes on swelling power is imperfectly represented by the maximum swelling because of their very different peptizing effect. The more they promote swelling the more of the gel do they send into solution. Posnjak has measured and compared the actual pressures and Katz the vapour pressures of gelatin gels under various conditions. Swelling pressure varies as a power of the concentration ($P = P_0 C^k$) P_0 being a constant and k having a value of almost 3. For a 30 per cent gelatin gel the pressure is about 500 grams per square centimeter and for a 60 per cent gel about 5000. The lyotropic series is evident especially among anions. Compared with pure water they promote (or depress) swelling in the following order in concentrations of 0.5 M or over:

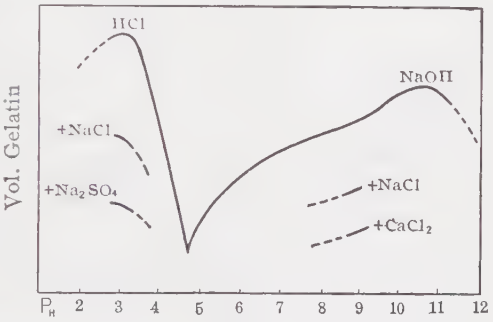

Fig. 50.

$$\text{CNS} > \text{I} > \text{NO}_3 > \text{Br} > \text{Cl} > \text{H}_2\text{O} > \text{acetate} > \text{sulphate} > \text{phosphate}$$

5. Dispersion and Coagulation of Emulsoids.—The stability of emulsoids is partly electrical as shown by the fact that like suspensoids they are most easily precipitated at their isoelectric point. It is to a greater extent due to hydration or the tendency to go into solution, so that, even when isoelectric, they remain stable unless acted upon by some powerful dehydrating agent such as alcohol or concentrated salt solutions. Precipitation by the latter is termed **"salting out."** This differs in principle from the purely electrical precipitation of suspensoids by dilute electrolytes. In " salting out " the effect of the two ions is additive and depends mainly on their dehydrating power. Thus the lyotropic series is pronounced, salting out efficiency being as follows:

Anions: citrate''' > SO_4'' > HPO_4'' > acetate' > Cl' > Br' > I' > CNS';

Cations: Li' > Na' > K' > NH_4' > Rb' > Cs' > Mg.

Coagulation through salting out is reversed by dilution of the salt. The mechanism of salt action is more complex than mere competition for water, as is shown by the fact that CNS' and I' tend to peptize instead of coagulating and that in acid the *order of the series is reversed.* Adsorption of the electrolyte also comes into play as described below.

The effect of *more dilute electrolytes* (below .5 M) on the dispersion of emulsoids is best studied on those imperfectly lyophilic colloids such as globulin and denatured albumin which show instability near their isoelectric point. Starting with the unstable, more or less neutral, colloid and adding electrolytes, we find that peptization becomes more and more pronounced as electrolyte is added up to a certain concentration. The peptizing power increases with the valency of either cation or anion. The colloid takes the charge of the polyvalent ion. When both are monovalent, as in NaCl, the charge is negative (see Table 157 in Freundlich). Thus salts of $CaCl_2$, $LaCl_3$, etc., act oppositely from, or antagonize, NaCl, etc.

If we consider the lyotropic series of ions we find that in *dilute* solution the *peptizing* order is the same as the *salting out* order in more *concentrated* solution. The more hydrated ions peptize best, probably because in virtue of the high ratio of adsorbed to free ions water is attracted to the particles. Presumably both ions are bound, the anions in excess. As an example of the powerful antagonism of Li salts in peptizing concentration to the flocculating action of polyvalent cations, the following table from Freundlich is included.

Cation Antagonism with Hydrophile Sulfur Sol

Concentration of $\frac{Li_2SO_4}{2}$ (First Added) In Percentage of Flocculation Value in Pure Solution	Flocculation Value of the Second Electrolyte		
	$MgCl_2$	$BaCl_2$	$CaCl_3$
0	100	100	100
35	2000	235	8,000
65	2300	160	15,000

Unlike their behaviour in the coagulation of suspensoids equivalent ions show large specific differences depending presumably on hydration—where hydrophile colloids are concerned. Thus a measure of antagonism may appear between ions of the same valency—Na v. K or Mg v. Ba.

6. Action of Emulsoids on Suspensoids. (*a*) *Sensitization.*—Two oppositely charged colloids may precipitate one another even if one is emulsoid. In other cases the emulsoid merely renders the suspensoid more sensitive to electrolytes, probably by partially discharging it. This happens only with low concentrations of emulsoid.

(*b*) *Protection.*—In excess the emulsoid usually protects the suspensoid by forming a film over it. Protective colloids have long been used for keeping insoluble substances artificially suspended, and in nature they play an important part in the same rôle. The "potable gold" of the ancients is a very old example; the stability of a mayonnaise dressing (due to egg-white) and the velvety texture of ice cream—when the ice crystals are isolated by gelatin—are more modern instances. In living nature, latex globules, fats, casein, minerals, etc., are prevented from flocculating in the same way. "Gallstones" and similar pathological concretions may be due to lack of hydrophilic stabilizer.

The relative protecting power of different proteins is sometimes used to distinguish them. Globulins are the most and albumins the least protective to a gold sol. Some emulsoids even have a peptizing power as illustrated in the detergent action of soap.

7. Elasticity and Rigidity of Gels.—Although dry gelatin, of course, does not stretch so easily as wet gelatin, there comes a stage in hydration when further swelling increases instead of decreases the elastic resistance to deformation briefly called rigidity. Figure 51 shows the reciprocal of the stretch under a given weight of a cylinder of gelatin jelly, in relation to *p*H. (The nature of the acid or alkali is of minor importance.) The rigidity curve

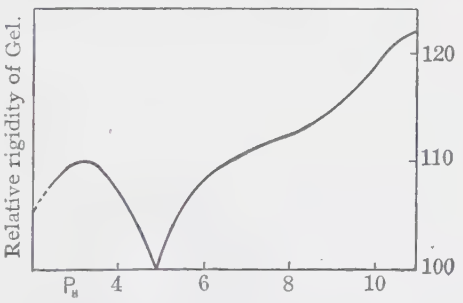

Fig. 51.

resembles the swelling curve except that the maximum in alkali is greater than that in acid. Similarly a reduction in rigidity takes place when swelling is reduced by salts.

8. Time Factor in Colloidal Phenomena. Hysteresis.—The swelling power, elasticity, etc., of a gel depend not only on its composition but also on its previous history. The effect of past history is termed **hysteresis.** Properly hysteresis means *lag* or delay in reassuming an equilibrium condition, but the term is also applied to cases where the change is permanent. For example, if a strip of gelatin gel is stretched for a short time and then released the immediate recoil does not restore it to its original length. The final stage of recovery is slow, and even when the former size is reached the swelling power and elasticity still remain different from the original. Such hysteresis can be interpreted only as the result of structural modification and is one of the best proofs of structure in gels.

Ageing of colloids.—Most colloidal changes such as flocculation, peptization, swelling, etc., take a considerable time to complete, but even when equilibrium is apparently reached very slow changes continue which are sometimes described as "ageing." In sols this usually takes the form of a gradual coarsening of the particles; in gels of **syneresis**—shrinkage with expulsion of water—which is probably due to the same underlying cause. A more rapid syneresis frequently accompanies coagulation.

9. Colloids in Serology.—The complex relationship between certain reacting substances in blood serum seems to become more intelligible in the light of colloidal behaviour. Evidence that the immunity conferring materials which are produced as a result of infection undergo *adsorption* by the bacterial cells or by their toxic products was mentioned earlier (p. 58). It remains to point out how the laws of *colloidal precipitation* are followed in seral reactions. The colloidal nature of the reacting substance is amply proved by dialysis, etc. The action of *agglutinins*, as already stated, is to "sensitize" the bacteria to precipitation by Ca^{++} and other active cations. The sensitization in this case appears to involve a reduction of hydrophily rather than of charge on the bacterial surface. Also, agglutinins show their maximum effect in an intermediate concentration, just as mutually precipitating colloids often remain stable in excess of either. Similarly, minute amounts of viperine venom coagulate blood while larger

amounts fail to do so. The reaction between *toxin* and *antitoxin* presents a further analogy to colloidal precipitation in its dependence on the mode of mixing. If a concentration of antitoxin which, when added in one instalment, is able to neutralize a certain toxic serum, be added in successive stages to the same serum, it may fail to abolish its toxicity. This is analogous to the case where gradual addition of a certain concentration of electrolyte fails to precipitate a colloid while sudden addition does so—perhaps because there is less even distribution of ions to particles.

IV. SUMMARY OF COLLOIDAL ACTION OF ELECTROLYTES

1. Action on Suspensoids. The principal colloidal change is in the matter of dispersion. This depends on electrical charge, and electrolytes modify dispersion through modification of charge. If the colloidal particles bear a considerable charge to begin with, it is always the ion of opposite sign which is manifestly active, adsorption being predominantly electrical. If the colloid is ampholytic and near its isoelectric point, either ion, depending on which is mechanically adsorbed to the greater degree, may impart its charge to the particles. In the former case addition of electrolyte usually decreases charge up to a point and in higher concentrations sometimes reverses it. In the latter case electrolytes increase charge up to a certain concentration and beyond that cause it again to decrease.

2. Action on Emulsoids.—The observable modifications which are important in emulsoids include not only visible changes in dispersion but also changes in viscosity and osmotic pressure (of sols) and swelling and rigidity (of gels), all of which depend both on the electric charge and on the hydration of the particles. As far as charge is concerned the same rules apply as to suspensoids.

Thus in dealing with proteins, for example, if they are strongly charged either by acid (+) or alkali (−) the important factor in the influence of a not too concentrated salt is the valency of the oppositely charged ion, because here adsorption is mainly electrical and the chief variable is the charge on the particles. Even though the *p*H is kept constant the charge is reduced because the isoelectric point is brought nearer.

Hydration of particles, though influenced by charge, also increases with the hydration of the adsorbed ions or molecules and

tends to decrease with the hydration of ions or molecules remaining in solution, but it is only in rather high concentrations of salt that the hydration of the ions becomes a prominent factor in the above case.

If the protein is *isoelectric* to begin with, both ions tend to be adsorbed and, therefore, hydration varies more than charge and the hydration of the ions is of greater moment. Only if one ion is of higher valency or, decidedly more adsorbable than the other do we get much charging effect, which, as always, falls off in higher concentrations of the electrolyte. The maximum charge (and also dispersion and hydration) usually reaches a higher level and does so at lower concentrations of electrolytes, the greater the valency or adsorbability of the active ion. H and OH are particularly active. With monovalent salts the anion is slightly more adsorbed than the cation but apparently both are attracted since the hydration of the particles increases with the hydration of the cation as well as of the anion.

If the protein is near the **neutral point** to begin with, the behaviour is intermediate between those of the above two cases. When dispersed in water, proteins have more or less negative charge depending on the position of their isoelectric point; consequently active cations reduce charge or in higher concentrations reverse it. Salts of monovalent cations, unlike their action on suspensoids, do not in dilute solution reduce charge, at any rate not to an appreciable degree. Both ions are adsorbed and both confer their own hydrophile property to the colloid, which, therefore, tends to show increased dispersion, viscosity and swelling and a reduced tendency to be flocculated by polyvalent cations.

Another key to the complex action of electrolytes on emulsoids is the fact that every electrolyte attains both a maximum of charging effect and also of hydrating effect at certain definite concentrations, and just as this concentration of maximum charging effect is lower the greater the charge or valency of the adsorbed ion, so approximately is that of maximum hydrating effect lower the greater the hydration of the ion or ions. Thus, whereas in high concentrations (M 5 and over) sulphates and chlorides have passed their maximum hydration point, thiocyanates and iodides are still in the hydrating and peptizing zone of concentration. Similarly, at a somewhat lower concentration occurs a turning point for the cations, when Li, for example, from being the most

hydrating, becomes the most dehydrating of the alkali metals. This applies to negative colloids.

The fact that the *most hydrated* ions are also the *least adsorbable* helps to explain why at this and of each series the effect of the ions in solution soonest overcomes that of the ions adsorbed as the concentration is increased.

The actual concentration at which the maximum of charge or hydration, respectively, is produced varies with colloid as well as with the electrolyte. In the case of agar, for example, it is so low that there is usually enough present as impurity to give maximum swelling in water.

3. Antagonism of Ions.—There are three modes of antagonism of electrolytes:

(1) The effects, either on charge or hydration, are opposed, and in combination one is subtracted from the other.

(2) The action of the simple electrolytes is the same. In combination the effects are added, and a turning point may be passed; for example, the concentrations of NaCl and $CaCl_2$ which produce maximum swelling singly, when mixed shift the conditions to the descending side of the curve.

(3) The electrolytes act similarly on charge but differently on hydration, and the hydrating effect of one opposes the discharging effect of both together, illustrated by the antagonism of Li and Na salts to the precipitating action of divalent cations.

Dispersion, osmotic pressure, swelling pressure and true viscosity all increase both with charge and hydration of particles. Rigidity, on the other hand, although within limits it increases with hydration, decreases notably with dispersion. Thus the apparent viscosity (or consistency) and gelling power of a sol, which depend largely on aggregation of particles, may be at a maximum at the isoelectric point, at which the other properties mentioned are at a minimum.

Reference to the relations here summarized will aid in an understanding of the complex responses of protoplasm to electrolytes.

CHAPTER XIV

COLLOIDS IN THE CELL

Protoplasm is a mixture or compound of proteins, lipoids and carbohydrates, all of which tend to be colloidal. Its notable viscosity (and even elasticity) in spite of high water content, and its ability to undergo marked changes in viscosity at a constant temperature are properties displayed only by emulsoid (hydrophile) colloids. The optical emptiness which its ground substance sometimes displays under dark field illumination is also proof of an emulsoid rather than suspensoid nature. On the other hand the rather low concentration of many electrolytes which suffice for its coagulation indicates a lower degree of hydrophily than is associated with typical emulsoids, and commonly also there are suspended in this clear substance granules and droplets of distinctly hydrophobic (lipoidal) nature. Therefore, in studying the colloidal properties of protoplasm we are concerned with those displayed by suspensoids as well as by emulsoids.

The phenomena to be dealt with may be classified as follows:

Adsorption
Dispersion and flocculation
Viscosity and consistency
Swelling (of gels) and osmotic pressure (of sols)

Adsorption has already been treated at some length. In addition it enters into all colloidal phenomena about to be described.

I. STATE OF DISPERSION

As in physical colloids so also in protoplasm, alteration in the state of aggregation may be either irreversible or reversible.

1. Irreversible Aggregation.—One type of aggregation, irreversible except through a digestive process, is the separation out from the mass of the protoplasm of a more or less pure substance

in the form of cell membranes, food bodies or excreta. These separated substances, for example, pectin, cellulose, starch, protein, oil, mineral deposits, etc., may be regarded as dead products of the living substance. We mention them merely because their condensation in some cases depends upon H-ion concentration in much the same way as does the aggregation of colloids, that is, by showing an optimum at what may be their isoelectric point. Freshly formed zygospores of *Spirogyra* plasmolyzed at various *p*H's for four days showed the following result as regards formation of a new spore wall:

Medium: *M*/2 Sucrose in Ditch Water +	*p*H at End	Zygospores with New Wall
M/8000 Acetic Acid	4.5	None—some dead
M/16000 "	5.5	All
M/32000 "	6.2	50 per cent
0	7.0	Only odd cells

Similarly the deposition of starch in guard cells takes place only within a narrow *p*H range (approximately 4.8 to 6.0).

Irreversible coagulation of protoplasm itself resembles that of albumin. Under the ordinary microscope the clear substance is seen to become gray and granular. Under the ultra-microscope the earlier stages can be observed: the progressive appearance, growth and flocculation of light-scattering particles, just as in the denaturing and flocculating of an albumin sol. At the same time the substance changes from the liquid state to a solid coagulum. Syneresis usually follows; the protoplast and its organs shrink and assume an irregular outline. In fixing for histological work this is avoided as much as possible by using the most rapidly acting coagulants, but, even so, some structural artifact is inevitable.

The resemblances to albumin coagulation extend further. The agents which cause coagulation of protoplasm also denature and flocculate albumin, for example, chemical agents such as (1) *colloidally active cations* particularly noble and heavy metals, trivalent ions, the hydrogen ion, and some organic cations; (2) also, in acid solution, *colloidally active anions*, e.g. chromate, osmate, picrate, etc.; (3) *capillary active non-electrolytes* (narcotics); and such physical agents as *heat* and *ultra violet light*. Even mechanical

coagulation of protoplasm can be faintly imitated in the denaturing of albumin. Protoplasm, however, is more sensitive to most agents than is albumin. Below are Lepeschkin's figures for the percentage of concentration of various narcotics which completely coagulate albumin within ten minutes, and also those which coagulate the protoplasm of yeast cells (the figures for albumin are theoretical, calculated from the coagulation value in presence of 24 per cent alcohol).

	Ether	Chloroform	Benzol	Thymol
Albumin	11.9	5.1	2.0	1.3
Yeast cells	4.5	0.7	0.2	0.06

The sensitiveness of protoplasm is greater in each case, increasingly so with the apolarity of the agent. Electrolytes, also, coagulate protoplasm in lower concentration than is required for albumin. Whether the lower degree of hydrophily results from the nature of the protoplasmic proteins or from their being combined with lipoids is not known.

2. Reversible Changes in Dispersion.—The most easily observed dispersion changes are those of granules and droplets of visible size whether in the cytoplasm or cell sap. These are by nature more hydrophobe than the matrix and respond readily to dilute electrolytes which can enter the cell, for example, basic dyes, and organic acids and bases. The behaviour with these has already been described under "Adsorption." It appears that many of the granules are amphoteric, flocculating reversibly in an intermediate *p*H range, about 5 to 6. Others such as latex globules do not redisperse in higher concentrations of acid.

Reversible changes in aggregation of colloidally dispersed substances of the cell also take place:

(*a*) The vacuole of many plant cells contains colloidal material in optically homogeneous solution, which may be precipitated reversibly by basic dyes. The precipitate redisperses both in alkali and in acid. In other cases, especially if tannin is present in the sap, weak bases and alkaloids precipitate and acid redisperses the colloidal complex. The precipitate usually takes the form of droplets which tend to fuse (Fig. 52).

(*b*) The clear ground substance of the cytoplasm apparently contains elements which go to enlarge the visible granules when these are flocculated by dyes, etc. (see p. 63). Also, incipient coagulation of the matrix itself by colloidally active cations—detected ultramicroscopically by the development of a milky appearance—is reversible if not carried too far.

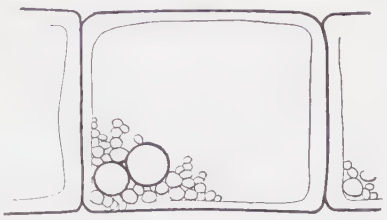

Fig. 52.

(*c*) In the nucleus, aggregation and dispersion of chromatin are a feature of mitosis, and can likewise be induced experimentally by acid and alkali respectively. As regards the condensation during mitosis it is suggestive that cytoplasm (*p*H 6.9) is more acid than the nucleus (*p*H 7.5) and that as the nuclear membrane breaks down the nuclear *p*H may, therefore, fall.

3. Periodical Precipitation in Organisms.—The fact that the precipitate resulting from the diffusion of one inter-acting solute into a solution of another is frequently zoned was referred to above (p. 161). It is one which the biologist must keep in mind, first because in applying reagents to tissues he frequently obtains the same result, and second because it offers a relatively simple explanation of many naturally occurring banded markings. As an example of the former, may be mentioned the forms of precipitate obtained when cobalt sodium hexanitrite, a reagent for potassium (Macallum), is allowed to diffuse into tissues. Striking bands are obtained in nerve fibers, plant hairs, and various other structures. On account of a fatty sheath around the nerve the reagent is able to penetrate only at certain points (nodes) where this is interrupted. The progressive widening of the bands of precipitate from these points along the nerve shows that this is simply a Liesegang phenomenon. Similarly plant hairs, being covered by cuticle, admit the reagent only by way of their base. Proceeding therefrom the spacing of the bands widens toward the apex, with complications if septa are present. The formation of bands in such cases is no indication of a corresponding localization of potassium in the tissue but only of the route of entry of the reagent (Fig. 53).

Examples of concentric markings or layering in organic bodies are numerous. Many of these, for example, annual rings in trees

and daily layers in cell walls and starch grains, are the product of an external periodicity; but others seem to depend on an internal rhythm, which in some cases may be of the nature of a periodic precipitation. The fine concentric markings in many feathers, the regular bands on certain leaves (*Sansevieria*) and even the stripes of the zebra are possible examples, because we must remember that the condition originated in the embryonic state of the organ or animal, when the size would suit a diffusion phenomenon. It must be added, however, that definite proof of the theory is still lacking.

Fig. 53.

II. VISCOSITY AND CONSISTENCY

1. Interpretation of "Viscosity."—The difficulty of interpreting "viscosity" measurements in emulsoids has already been referred to, and applies with especial force to protoplasm which is more heterogeneous than a simple sol. Protoplasm is in fact generally a visco-elastic system, neither purely liquid nor purely solid. Certain criteria such as the Brownian movement or displacement by centrifuging of small particles give information mainly as to viscosity, whereas others, such as resistance to change of shape and recovery of shape depend also on *rigidity* and *elasticity* respectively. A method which has been applied to animal tissues, such as the effect of the tissue on the amplitude and frequency of vibration of a spring attached to it (Gasser and Hill; Hogben),[1] seems to allow the two factors, viscosity and rigidity, to be discriminated; but generally what is observed is a combination of the two which may be termed *consistency*, the reciprocal being *plasticity*.

2. Methods of Estimation.—The methods which have been most used to estimate consistency are: (1) the displacement by centrifuging of particles or organs of the cell; (2) micromanipulation; (3) observation of Brownian movement; (4) of the behaviour during plasmolysis; (5) of the tendency to assume minimal area; and (6) of the rate of plasma streaming. Most of these are

[1] Gasser H. S. and Hill H. V., Proc. Roy. Soc. B. 96 p. 398 (1924) Hogben, L. T., Brit. J. Exp. Biol. 4, 196 (1926).

available to the ordinary student, at least for the purpose of qualitative comparison.

The relative amplitude of Brownian movement is perhaps the most easily applied test. Notable changes in this respect can be observed in amoeboid cells. The agitation of particles is rapid, for example, in the interior as compared with the cortex of an ordinary amoeba and in regions where a pseudopod is about to protrude as compared with quiescent parts. The latter is very strikingly exhibited by leucocytes under the ultramicroscope. Motionless granules suddenly spring into rapid oscillation prior to and in the region of the extension of a pseudopodium.

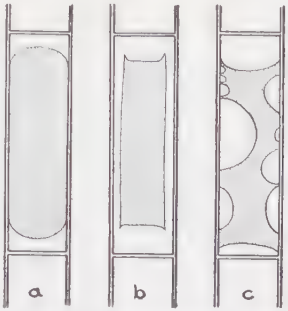

Fig. 54.—Plasmolysis forms, diagrammatic. (*a*) 'Convex,' (*b*) 'Angular,' (*c*) 'Concave' (after Weber).

The behaviour during plasmolysis gives information as to the consistency of the plasma surface and sometimes of the interior. Uneven separation (" concave " plasmolysis) as compared with smooth (" convex " plasmolysis) may indicate a higher consistency of the surface, but it may also result merely from greater adhesion between protoplasm and cell wall as shown by the fact that from other tests viscosity may be lower, not higher. " Angular " plasmolysis where the protoplast tends to preserve its original shape probably bears more relation to the physical state of the protoplast as a whole. The *rate* of rounding off by the protoplast may also be used as an index. In certain cases the plasmolysis shape depends also on the consistency of internal organs of the cell, for example, the chloroplast of *Spirogyra* (" spiral " plasmolysis).

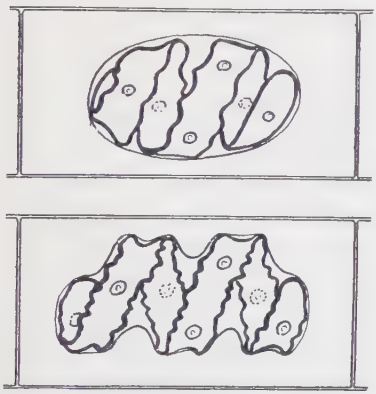

Fig. 55.—Plasmolysed cells of *Spirogyra*. Above—chloroplast plastic—'convex' plasmolysis. Below—chloroplast more rigid—'spiral' plasmolysis.

The tendency of protoplasmic surfaces to assume minimal area

is determined by surface tension as well as by plasticity and as a test of viscosity must, therefore, be checked by some other test. Thus the reduction of surface of the chloroplasts and cytoplasmic strands of *Spirogyra* may be shown by the plasmolytic and centrifuge methods and from Brownian movement to be due partly at least to lowering of viscosity. The same need for a counter check applies to the rate of plasma streaming as an index of physical state.

The rate of fall under gravity or centrifugal force of bodies (granules, starch, etc.) within the cell which have a higher specific gravity than the matrix, has been employed to give even quantitative values for protoplasmic consistency. Those given for mature plant cells have little significance because the layer of cytoplasm is extremely thin and only a small area of the plastid which envelops the large starch grain (and moves with it) is in contact with cytoplasm at all. Heilbrunn's measurements, by the centrifuge method, on sea urchin eggs, indicate a rather low viscosity of the interior of such cells.

Micromanipulation of protoplasm by fine needles shows that it is often more viscous or gelatinous than we might suppose from its capacity to flow and change shape. Probably the magnification of the microscope gives an undue impression of the latter.

3. Influence of Chemical Agents. (*a*) *Electrolytes.*—The effect of reduction of charge as we have seen is complicated by its double action—dehydration leading to decrease of viscosity and aggregation which may lead to increase of rigidity. The results as regards " consistency " may thus be either rise or fall according as to which of the above changes predominates. Taking account of this anomaly it may be said that the behaviour of protoplasm is what one might expect if the action of electrolytes is a direct colloidal one. A shift of the protoplasmic *p*H in the alkaline direction increases viscosity, slight acidification usually decreases it though gelation may ensue. Higher concentrations of acid cause irreversible coagulation. CO_2 acts like other acids. Salts of the alkalis applied externally tend to liquefy and disperse the plasma surface—often though not always in the order: Li > Na > K. (Their hemolytic activity is: K > Na > Li.) After they have had time to penetrate, these ions may at first increase viscosity (Heilbrunn)—no doubt by increasing hydration—but as swelling and peptization proceed liquefaction follows. Microinjection of

solutions of the above liquefies both the granular protoplasm and plasmalemma of amoebae in the order Li > Na > K.

Salts of bivalent ions when applied externally tend to increase slightly the adhesiveness of the protoplasmic surface. The effect on its consistency is doubtful, but there is notable antagonism to the dispersive action of the alkalis. Trivalent salts increase both adhesion and consistency. Among polyvalent cations Ca and some others often fail to produce any internal effect, but some of the more penetrating (Ba, La and heavy metals) produce a lower viscosity at first (see experiments on *Spirogyra*), followed later, in higher concentrations at least, by gelation. The concentrations which may produce the fall of viscosity have a very low limit (10^{-5} or 10^{-6} *M* or even less). It is apparently a true " electroviscous effect." How salts of polyvalent cations act when injected into the cell in low concentrations is uncertain; in higher concentrations solidification is the result (Chambers and Reznikoff). Here again the peptizing action of the alkalis is counteracted, although, as with physical colloids, the order of antagonism is not always easy to explain in terms of the potency of single salts. Thus K is antagonized better in its action on the internal protoplasm and Na in its action on the plasmalemma. Not only does the relative activity among themselves of monovalent and divalent ions vary with the part of the cell, but also as regards the same part with different cells, indicating a variation in composition with the species.

Anions have much less effect than cations on protoplasmic consistency—as is usual with a negative colloid—and such effects as have been recorded are not properly distinguished from possible H-ion changes.

(*b*) *Narcotics.*—These tend to dehydrate protoplasm and produce at first a fall in viscosity. The result of this dehydration, however, is that the protoplasmic colloids become more sensitive to the electrolytes present in the cell and that therefore gelation and coagulation usually follow.

III. SWELLING AND OSMOTIC PROPERTIES OF CELL COLLOIDS

1. Swelling Pressure v. Osmotic Pressure.—It is impracticable to separate the swelling pressure (imbibition pressure) of protoplasm as a gel from its " osmotic " pressure as a sol, and we shall

consider both together as swelling pressure. To form an estimate of the part played by cell colloids in turgor we need some idea of the degree of pressure set up by hydrophile gels of similar dry weight concentrations. As a basis of comparison let us take the swelling pressure of gelatin at room temperature:

18.6 per cent concentration	0.3 atmospheres pressure	Northrup
30 per cent concentration	5 atmospheres pressure	Northrup
50 per cent concentration	30 atmospheres pressure	Freundlich
60 per cent concentration	50 atmospheres pressure	Freundlich

Protoplasm in active cells has a dry weight concentration of 10 to 35 per cent; therefore, even if it equaled gelatin in hydrophilic property—which it probably does not—it would only be at the higher concentrations that swelling pressure could be the chief agent in the total pressure of 5 to 7 atmospheres common in cells. In the thin layer of cytoplasm in plant cells, however, swelling pressure must be the major factor determining volume, and in most animal cells it is a not inconsiderable factor.

2. Swelling Pressure and Retention of Water.—In specific cases, however, swelling pressure becomes of supreme importance in capturing or retaining water for the cell. The most insistent peril that besets terrestrial life is loss of water either by evaporation or freezing. Among the many defences to prevent loss by evaporation the development of strong swelling capacity is the last and most impregnable resource. Plants of desert and seashore, where drought conditions may be intense, are commonly succulent due to the presence of much hydrophile colloid in walls and cell sap. Similarly in plants and animals which are adapted to resist extreme cold the protoplasm has unusual swelling pressure. More water is bound to the colloid particles than in less hardy plants. The most intense swelling pressure is shown by seeds and spores. "Dry" seeds will tolerate the lowest temperatures that can be produced—approaching absolute zero without loss of vitality. But when we consider that they will withdraw water from saturated lithium chloride (osmotic pressure 1000 atmospheres) the impossibility of freezing their protoplasm appears less strange.

3. Influence of Chemical Agents on Water Relations of Cells. (*a*) *Acid and Alkali.*—Cells and tissues bathed in solutions of graded *p*H often show minimum volume in a certain intermediate *p*H, either a point or region commonly around *p*H 4.5 to 6. The

*p*H of cytoplasm has been determined as about 6.9 in a great variety of cells and that of the nucleus as 7.5. Changes in the cellular *p*H produce changes in volume such as we might expect theoretically. Red blood cells swell in alkali up to a maximum and shrink in acid to a minimum. The accompanying table shows the relation of the volume of plasmolyzed protoplasts of *Spirogyra* in relation to the external concentration of penetrating acid and alkali. The zygospores swell more than vegetative cells, at least in acid. They are relatively free of cell sap.

Volume of Spirogyra Cells (Plasmolyzed with $M/2$ Sucrose in Ditch Water) in Relation to H-Ion Concentration

1–2 Hours Exposure

	Acetic Acid				H_2O	Ammonia			
Concentration.....	$\frac{M}{4000}$	$\frac{M}{8000}$	$\frac{M}{16,000}$	$\frac{M}{32,000}$	0	$\frac{M}{8000}$	$\frac{M}{4000}$	$\frac{M}{2000}$	$\frac{M}{1000}$
Approximate *p*H..	4.1	4.5	5.5	6.2	7.0	7.8	8.5	9.0	9.3
Volume vegetative cell.....	112	106	102	98	100	102	108	118	130
Volume zygotes...	135	130	107	100	100	100	105	115	125

The *p*H of the sap vacuole is much easier to change than that of the protoplasm and its colloids show greater volume change *in vivo*. The guard cells of stomata show immediate and notable changes of turgor when their internal *p*H is varied either by penetrating acid or alkaline solutions or by CO_2 gas. The volume change takes place mainly in the vacuole, which is rich in colloid. Swelling is at a minimum around *p*H 4.8 to 5.0 (within the vacuole) and increases the more acid or alkaline the cells become—short of injury. In nature the *p*H varies with CO_2 content. Active photosynthesis reduces CO_2 tension until the cells may attain a *p*H of about 7. As in nature also hydrolysis of starch reinforces the turgor increase when cells are bathed both by alkali and by acid but a speedier change in the sap colloid appears in all these

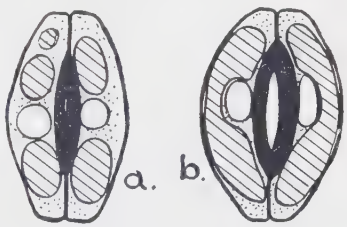

Fig. 56.

cases to produce the first effect. In some cases, root hairs, potato tissue, etc., more than one minimum is discovered in the relation of swelling to *p*H, indicating that more than one ampholyte is concerned.

Growth which is partly dependent on turgor sometimes shows a minimum and two maxima in the *p*H range. Lloyd found most rapid growth of pollen tubes in *M* 3200 penetrating acid and *M* 3200 alkali (NaOH) and minimum in distilled water. Later workers have found minima between *p*H 5 and *p*H 6 in the growth of fungi and seedlings. This relation may be the direct effect of *p*H on turgor, but the factors involved in growth are too complex to regard this simple explanation with assurance.

(*b*) *Neutral Salts.*—Salts of the alkalis in hyper- or isotonic concentration gradually penetrate the plasma membrane and cause the cytoplasmic layer to increase in thickness and become clear and highly fluid. Li salts have the greatest effect. Injected these salts act in the same way. Those of Ca, Mg, and other bivalent metals have the opposite action. The order of activity of the anions, apart from H-ion variation, is doubtful at present. The rôle of most of the salt constituents of a cell, such as chlorides and phosphates of potash, in the concentration at which they occur is probably a hydrating one, but Ca and Mg have the opposite effect. It is not only upon the concentration but also upon the balance of ions, of K and Ca especially, since Na is rare in cells, that the hydration of protoplasm depends. Factors which upset the normal concentration or balance of ions in cells lead sometimes to extraordinary changes in their water capacity. Pituitary extract in minute doses causes loss of salts (phosphates, K and Ca) from animal tissues, first into the blood and then into the urine. Following this the cells absorb much more than their normal amount of water (Stehle).

(*c*) *Effect of Narcotics on Cell Turgor.*—The influence of narcotics on cell turgor is complex and obscure. Their action on physical colloids is to reduce swelling both in water and salt solutions. In low concentrations they frequently have the opposite effect on cells if water is the medium, and regularly and markedly so if a salt solution is applied. *Spirogyra* filaments which have been exposed for some time to 1 or 2 per cent ether solutions become much more crisp and curly (turgid) than normal, and if salt solutions (NaCl, KNO_3, $CaCl_2$, etc.) are applied,

even in hypertonic concentration, the cells swell greatly as do also the cell organs. Vacuolation also takes place. The effect of the salt is probably the result of its penetration, as other factors than narcotics may enable it to produce the same result. The effect of ether alone in raising turgor is hard to account for, unless it is that the capillary active agent displaces from adsorption substances which are osmotically active. In higher concentrations narcotics produce irreversible syneretic shrinkage of protoplasm, as we might expect.

4. Contractile Movements of Protoplasm. Although certain forms of amoeboid movement and perhaps streaming in plant cells are capable of explanation in terms of surface tension change at visible surfaces, most movement of protoplasm has a deeper seated origin and is associated with variations in viscosity which point to a colloidal change. This is true at least of typical amoeboid and also of muscular and ciliary movement. With respect to their grosser mechanisms these various types vary enormously; yet they are connected by so many transitional forms that a common underlying mechanism has commonly been assumed—vaguely assigned to the contractility of protoplasm. Before considering whether such an assumption is warranted we must have in mind a rough picture of the principal types of movement.

(*a*) *Muscular Contraction* results from shortening of fibrillar structures in the elongate living muscle cell or fibre. The fibrils in voluntary muscle are regularly arranged in segments or strata—hence the name "striped muscle." Actually the arrangement would seem to be that of two flat helicoids. The most pertinent fact of structure, however, is that in the middle region of each segment the fibrils are anisotropic and that this is the part which shortens. It is uncertain whether the volume of the anisotropic part changes on contraction but the weight of evidence is in favour of its shrinkage (Tiegs). The chemical agent which produces contraction is lactic acid (H ions). Neutralization of the acid causes relaxation. At rest the reaction of the cells is about neutral, that is to say, the proteins of the myofibrils are normally on the alkaline side of their isoelectric point, so that acidification, by bringing them nearer to that point, should theoretically cause the fibrils to shrink in volume. Their anisotropic structure is sufficient to account in a general way for the fact that this shrinkage is mainly, if not entirely, uniaxial. Not enough is known of the finer struc-

ture and molecular arrangement to define the mechanism more precisely than by analogy with the behaviour of other anisotropic gels, such as cellulose walls of plants. Attendant changes in the physical properties of the muscle substance during contraction support the view of the mechanism which is here presented. Muscle being a visco-elastic system can undergo change both in elasticity and viscosity. A gelatin gel is more extensible at its isoelectric point than at the neutral point — so is muscle. A protein sol is less viscous under the same condition — so, according to Hogben,[1] is muscle, as tested by Gasser and Hill's vibration method.

(*b*) *Contraction in Spirogyra.*[2] As an example of a contractile organ which is quite unspecialized for that purpose, save that it happens to be somewhat anisotropic, we may again consider the chloroplast of *Spirogyra*. This structure may shorten as a gel (Fig. 57), as well as under the moulding action of surface tension after it has liquefied as shown earlier in Fig. 14. Here too gel contraction is accompanied by a loss of rigidity. Contraction in this case is commonly the direct result of applied chemical agents and can be shown to be induced by many other colloidally active cations besides $H^{\cdot}$. This lends force to the theory that reduction of charge on certain particles or surfaces is the cause of contraction.

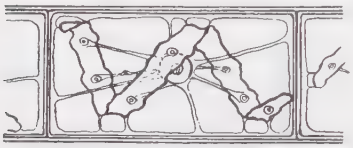

Fig. 57.

(*c*) *Amoeboid Movement,* according to the most approved theory, is also the result of gel contraction accompanied or succeeded by liquefaction. The outer layers (**ectoplasm**) of an amoeba are of much higher consistency than the **endoplasm.** In an amoeba moving limax fashion the whole of the ectoplasm forms a tube tapering toward the posterior end of the animal. In the posterior region the protoplasm of the tube liquefies and becomes endoplasm. It then flows forward and is converted into ectoplasm again anteriorly. The ectoplasmic tube when adhering to a substratum is

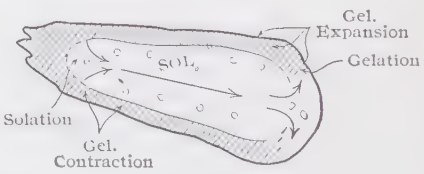

Fig. 58.

[1] Br. J. Exp. Biol. 4; 196, 1926.
[2] Q. J. Exp. Phys. 14; 99, 1924.

stationary. Relative to the animal as a whole, however, it moves backward and, therefore, on account of its tapering form must evidently contract. The theory is that this contraction is the motile force that causes the endoplasm to flow forward and produce the so-called pseudopodium. Analogy with muscle naturally suggests that we look for evidence in amoeba of a development of acid in the region of contraction and liquefaction but the experiments are still inconclusive.

Fig. 59.

(*d*) *Ciliary Movement.*—The rhythmic beat of a cilium, as Gray has demonstrated, is also accompanied by simultaneous alterations in rigidity. The cilium becomes flaccid at the end of its effective beat corresponding to the contraction phase in muscle. It is suggestive that just as accumulation of acid fixes a muscle in its phase of contraction (rigor), so acid arrests a cilium at the end of its effective beat.

(*e*) *Colloidal Mechanism of Contraction.*—A certain common element appears, therefore, in all these types of movement, namely, contraction of a gel structure and probably a fall of viscosity or rigidity, both of which may be produced by colloidally active cations such as $H^{\cdot}$, $Ba^{\cdot\cdot}$ and $La^{\cdot\cdot\cdot}$ in extreme dilution. While recognizing that protoplasm especially in the form of organs like striped muscle and cilia, adapted to contract with great speed and coordination, undoubtedly possesses a vastly more complex organization than any physical gel, we may as a working hypothesis regard the fundamental colloidal mechanism as similar to that which causes the *shrinkage of a gel when its micellae are discharged*, the uniaxial character of the shrinkage resulting from the anisotropy of the gel.

Reduction of charge on the colloidal surfaces means increase of effective surface tension as well as decrease of hydration. The gel shrinkage theory is, therefore, not opposed to that form of the surface tension theory of contraction which ascribes the result to decrease of charge (Tiegs) but it adds something to it, namely, the dehydration factor. The view is diametrically opposed to the imbibition theory of Engelmann which assumes, contrary to all indication, that the proteins are isoelectric at rest and swell when lactic acid is liberated.

IV. HIGHLY ORGANIZED COLLOIDS

Certain types of colloidal particle which are produced only in presence of living cells, but may continue to exist essentially unchanged apart from cells, exhibit in varying degree properties characteristic of life. The things referred to are *enzymes* and filterable *viruses*. The former admittedly are not alive. Whether or not the latter are animate is uncertain. But both exhibit a surprising degree of organization in view of their minute size, and a consideration of their properties will throw some light (theoretically at least) on the simplest possible forms of life.

1. Enzymes are organic, colloidal catalysts—substances, that is, which increase the velocity of a chemical reaction without themselves being used up in it. Inorganic catalysts are of two classes, those that act in solution (such as acid in hydrolysis of cane sugar to grape sugar) and those that act as a distinct phase with surfaces capable of adsorption. Enzymes are of the latter type. As was mentioned in dealing with adsorption, this type of catalyst is probably effective partly through the concentration and orientation of the reacting molecules at its surface, and partly through the more specifically chemical mediation of components of its surface, as exemplified by the effect of traces of iron in the catalysis of organic substances on charcoal. The chemical factor involves a greater specificity than belongs to mere adsorption.

In the case of enzymes this specificity is carried to an extreme degree. Both within the cell and in the test-tube chemical reactions of innumerable sorts are promoted by distinct enzymes. Most of them catalyze only one reaction or a group of allied reactions. They show that the lack of specificity which characterizes the relations of inorganic colloids as compared with smaller chemical units is not inevitable to their size, but depends on the way the particle is built up or organized (which is especially interesting since specificity is so prominent in life). Thus, although enzymes may act dissevered from the organization of the cell they display a high organization of their own. This is also illustrated by the readiness with which they are modified as regards both action and character. For example, their catalytic activity is greatly influenced by H-ion concentration and by temperature. With temperature this activity increases up to a point

beyond which it rapidly drops because the enzymes as such are destroyed. The limiting temperature is in the neighbourhood of only 60° C. In these and other characteristics, such as their sensitivity to various "poisons," enzymes show resemblance to living matter. The principal distinction is that they do not reproduce themselves.

2. Filterable viruses are bodies of colloidal but apparently very variable size, which cause various diseases in all kinds of organisms from bacteria to man. They are called *filterable* because they pass the finest filters, such as hold back bacteria, and are called *viruses* because it is not known whether they are organisms or merely poisons. At first sight it might seem obvious that they must be organisms because they multiply, but it is still doubtful if there is a single case where they do so in absence of living cells. Thus there are two possibilities: (1) that one virus is produced directly from another but, being an obligate parasite, only in presence of living cells; and (2) that they do not reproduce directly but act as auto-catalysts. Just as there are certain chemical substances which catalyze a reaction that ends in their own production, it may be that viruses are produced by reactions that take place in living cells or in their vicinity, reactions that require the presence of the virus itself to start them. Evidence from other properties of viruses is still insufficient to decide between these alternatives though points in favour of their vitality are accumulating.

It has been shown, for example, that viruses undergo change, sometimes irreversible, with change of host. Smallpox virus after passing through several calves becomes vaccine virus, and in time loses the ability to revert to smallpox in man. This suggests "adaptation" to environment and "mutation" in a biological sense, and, therefore, life. Also plant viruses which are carried by insects are sometimes highly specific in their "carrier" and may require to spend a period in the vector insect before they can be transmitted as a source of disease. In these and other respects, viruses behave like bacteria.

The critical question, however, is whether one "generation" of virus is split off from the antecedent one. If we suppose for the moment that this does happen, it will be interesting to inquire by what physico-chemical mechanism such a characteristically vital phenomenon as reproduction could take place automatically in a colloidal micelle. It has been suggested that exactly the same type

of process that is supposed to underlie ordinary catalysis may, in appropriate circumstances, result in self reproduction.

In ordinary catalysis by enzymes substances become attached to the surface of the particles owing to the electrical forces residing there and, as a result of some redistribution of forces induced from the contact, they break away again in a different form or in different combination from the original. The hypothesis is that one possible combination of appropriate adsorbates is a chemical unit like the enzyme itself and that if this were formed and broke away, just as other catalytic products do, the result would be reproduction. According to this view viruses are enzymes which catalyze a *synthetic reaction* resulting in their own production.

The other view of viruses is that they catalyze a *decompositon* of some element of the protoplasm which results in their own production. One must admit that there is more physical analogy for the latter. We know of cases where a substance (termed autocatyst) catalyzes its own production by hydrolysis—for example, the transformation of ethyl acetate plus water into ethyl alcohol plus acetic acid is hastened by the presence of the acid. But there appears to be no example of autocalytic synthesis. The answer which is given by the protagonists of the former view is that of course, nothing like this happens in non-living matter because, if it happened, by definition the matter would practically have attained the level of life. The hypothetical primal living unit according to Alexander and Bridges is a particle capable of carrying on ordinary continuous catalysis (either analytic or synthetic) on part of its surface and reproductive catalysis on another part. A colloidal particle or even a large organic molecule might conceivably satisfy these requirements.

Whether viruses are simpler or more complex than this hypothesis assumes, they form a suggestive link between typical living and non-living types of organization. Their plane of organization is no higher above that of physical molecules than is the organization of the ordinary cell above that of a virus, or the organization of an animal including man above that of his cells. There is mystery enough in all this ascending scale but no greater mystery or advance in the origin of life than in the evolution of higher from lower forms of life.

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Part II

LABORATORY EXERCISES

TO THE STUDENT

Here are not only facts to learn but problems to solve. Some of the experiments which follow may seem simple in the extreme, but, like the traditional falling apple, they have provided thought for great minds. Awkward questions are put concerning them intended to elicit not merely a search of the text or an appeal to an instructor but painstaking observation, contemplation and deliberation on your part. Says Gilbert in "The Gondoliers."

"Quiet, calm deliberation disentangles every knot."

Lest this authority seem frivolous we append a more classical tag (from Virgil's Georgics).

"Felix qui potuit rerum cognoscere causas."

Outline I

STRUCTURE OF CELLS: PROPERTIES OF PROTOPLASM

1. Symphoricarpus: *Cells of the Fruit.*—Many fruits contain large cells easily isolated. Examples: Symphoricarpus (Snowberry), Tomato, Apple. We select cells from the mesocarp of the Snowberry. Mount a little of the pulp in *M* 4 cane sugar. Note the irregularly shaped cells which easily become detached from one another. Some will have been injured (ruptured) by manipulation and become dead. Note crinkled cell wall and altered appearance of contents of such cells.

Select a living (turgid) cell which shows a nucleus and protoplasmic threads and study with high power. By focusing on the various "optical plane sections" throughout the thickness of the

cell obtain a conception of its three dimensional structure. Make a sketch.

Identify:

Nucleus: Shape? Homogeneous or granular in living cells? In dead cells? It includes the nucleolus, a highly refractive body, that is, with a well-marked outline. Situation of the nucleus in the cell?

Cytoplasm: Granular but with clear colourless ground substance. Localization—surrounding the nucleus, forming strands or a network connecting nucleus with periphery and forming a thin continuous layer beneath the cell wall.

Vacuole: Occupying the rest of the cell, clear and colourless. Smaller vacuoles may sometimes be seen as bubbles in the protoplasm. If the concentration of sugar outside is increased to M/3 or M/2 the vacuole loses water and contracts along with the cytoplasm (= plasmolysis).

Cell wall—very thin, covering the whole. Observe after staining with Janus Green (see below).

Properties of the Living Protoplasm.—Shows Brownian movement of particles, frequently streaming movements, and is continually changing its configuration; therefore liquid. Pulls out into long threads; therefore viscid. Hard to see, therefore refractive index near that of water. Other properties? Compare dead protoplasm.

Vital Staining.—Allow cells to lie for some time in very weak neutral red and also Janus Green. What is stained by the respective dyes in the living cell? In the dead cell?

2. Spirogyra, preferably a large-celled species. Study cell structure carefully as before and draw. Note form and position of nucleus, chloroplasts, protoplasmic threads, etc., and their relation and contact with one another. Do any causes suggest themselves for the shapes and positions assumed? With high power and oil immersion objectives study the clear cytoplasm between the chloroplasts identifying granules and kinoplasm (mobile?). What is the appearance of the surface of the central vacuole when seen in profile?

Vital staining as with Snowberry may be performed but will receive fuller attention later.

3. Elodea.—Study and sketch the architecture of the marginal cells of a leaf. Position and true shape of chloroplasts and

nucleus? Study movement. How are the chloroplasts transported? Does the whole cytoplasm or only kinoplasm stream? Do the chloroplasts move *en masse* or in linear series?

4. Tradescantia (staminal hairs).—Study cell structure and cytoplasmic streaming. Do the strands move *in toto* or only in part? Are they always taut or sometimes slack?

5. Nitella.—Note three regions in the cytoplasm.—(*a*) stationary outer layer with elongate chloroplasts spirally arranged, indicating some sort of structural organization in this layer; (*b*) inner layer without chloroplasts and streaming *en masse*; (*c*) stationary inner strips with scattered rounded chloroplasts.

6. Vaucheria.—Cut across filaments, or if possible slit open the end of one (Fig. 1). Mount under coverglass and squeeze with needle while examining with low power. Follow with high power. Does protoplasm exude as fluid? Does it mix with water, coagulate, or form droplets? (Some droplets should be observed, green with contained chloroplasts.)

Nitella or *Hydrodictyon* may be experimented with in the same way.

7. Paramoecium.—Mount in China Ink. What evidence do you find of a tougher cortex and more fluid interior? Ciliary currents, as revealed by behaviour of the carbon particles, should be studied especially near the "mouth." Food vacuoles (shape?) enclosing carbon are pinched off from the gullet and carried around by the circulation of protoplasm. Two contractile vacuoles of complex organization beat regularly. *Paramoecium* illustrates the complexity of organization attained by some unicellular organisms.

With minimum of water under coverglass, squeeze gently with needle while examining and observe behaviour of protoplasm when expressed.

Outline II

SURFACE TENSION IN PHYSICAL SYSTEMS

I. STATIC CONDITIONS: FORCES IN EQUILIBRIUM

1. The Surface Film of Liquids.—Float a light and a heavy needle on clean water. Drop the needle horizontally, and avoid contact with greasy fingers. Can you suggest a reason for the non-wetting and non-sinking of the needles (air-film?)? Touch the surface of the water with soap or with a finger which has been rubbed on the side of the nose. Explain the movement which follows and the partial or complete submersion of the needles. Does soap (resp. grease) appear to raise or lower the "tension" of the surface film?

2. The Principle of Minimal Area: Figures of Minimal Area.—Suspend a loop of fine thread in a soap film held in a larger loop of copper wire and puncture the film inside the thread. Or attach the loop of thread to the copper ring by three supporting threads and variously break the portions of film. Maximal area of gap means minimal area of film. Van der Mensbrugghe, 1866; see Bayliss.

Plane figures of minimal area are illustrated in the above experiment. Such curved figures as the funnel shapes produced as a wire ring is lifted from a soap solution, or that of a film suspended in a twisted loop will illustrate the principle that governs when the film is exposed to the *same pressure on both sides*. Compare the radius of curvature in two directions at right angles. Repeat the observation when pressure is unequal on the respective sides, due to tension of film acting on a closed space, for example:

(*a*) A bubble freely floating in air.
(*b*) The same uniaxially pulled out or compressed (hanging drop figures).
(*c*) One, two and three equal sized bubbles resting on the soap solution and in contact with one another.

(*d*) Unequal bubbles in contact. How does the internal pressure vary with the size of the bubble?

Why does a soap film not break up into drops—the true figure of minimal area—as water does? Compare threads of mucilage, etc. (viscosity?).

3. Measurement of Surface Tension by the Ring Method.—(*a*) *Construction of tensiometer* from the parts supplied, namely: *Wooden frame* as shown in the diagram, about 8 inches high, soft-wood upright. *Balance arm* consisting, for instance, of a strip of bamboo with small pieces of aluminum clipped onto each end and center, machine punched to a standard pattern. *Two small S hooks. Platinum wire* about 7 cm. *White metal chain* about 12 cm. *Thread*, 3 *pins*.

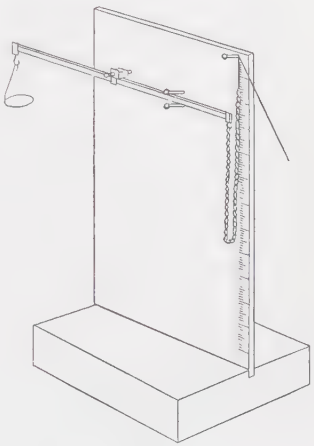

Fig. 60.

Insert hooks at each end of balance arm. Pivot on a pin inserted near left hand side of upright and about 2 inches from top. Steady the right arm of the balance by a couple of pins, one slightly above and the other slightly below it when in horizontal position. Hang 12 cm. of chain to right-hand hook and tie thread to lower end. Fix a graduated scale perpendicularly behind the hanging chain. Carefully measure 4 cm. from end of platinum wire and make sharp bend at that point. Bend the 4 cm. into a circular ring as flat as possible; make a small loop at the other end of the wire and arrange that the ring shall be horizontal when suspended by this loop from the left arm of the balance.

Cleanse the ring by heating in flame before every fresh test and do not touch with fingers.

(*b*) *Surface Tension of Water.*—Lift the free end of the chain by the thread attachment so that the loop of chain hangs perpendicularly from the end of the balance lever. Read off the height of the bottom of the loop when the lever is equipoised. (Alternately the position of the free end of the chain may be noted, with

the advantage that it moves twice the distance that the loop does.) Bring up a clean dish of fresh *tap water* until the liquid touches the ring. Lower the dish steadily with one hand and the chain with the other simultaneously (so as to keep the balance arm horizontal) until the film breaks. Repeat the make and break of film connection between liquid and ring until the height of the loop at the moment of break has been accurately determined. The difference in the two heights measured is the length of chain added to the right-hand arm to balance the tension of the film on the left-hand arm. Knowing the average weight of the chain (for instance, 0.072 gram per cm.) we can calculate the force applied in dynes: length of chain (l) $\times$ 0.072 $\times$ value of g (= 981). The surface tension of the water is equal to this force divided by twice the circumference of the ring (there being two surfaces), or 8 cm.

$$\text{Surface tension} = \frac{l \times 0.072 \times 981}{8}$$

(*c*) Touch the surface of the water with soap. Measure surface tension immediately and in quick succession for a few minutes. Explain the results.

(*d*) Compare the effect of inorganic salts on surface tension of water with that of the above and other organic compounds.

4. Interfacial Tension: Angle of Contact.—Surface tension at a liquid-liquid (oil-water or benzol-water) interface. Cover clean water in a clean dish with a layer of nujol about $\frac{1}{3}''$ deep. With the tensiometer measure (1) the interfacial (oil-water) tension and (2) that at the oil-air surface; then (3) that of water after contact with the oil. According to your results, should nujol theoretically spread on water? Test this experimentally. Note the gradual extension of area of a small drop placed on a clean water surface. Why does the oil spread so slowly? Cf. with warm water. (Effect of viscosity on spreading and of temperature on viscosity?) Compare the rate of spreading of grease from the skin by touching the water surface outside the area covered by the oil drop. Behaviour of drop? Compare surface tension of water surface before and after touching with finger. Should nujol spread now? As a further exercise the *triangle of forces* here involved may be plotted and from it the angle of contact between nujol and the greasy water surface determined.

[**5. Adhesion and Cohesion.**—The molecular forces which cause surface tension are concerned with other phenomena (cohesion, adhesion, solution) which are, therefore, to be related to surface tension.

(*a*) *Adhesion and Wetting.*—Is clean glass wet by water? By mercury? Paraffin by water? Try by dipping solid into liquid Compare adhesion of liquid to solid in each case. Is the fluid lifted against gravity? Rise in capillary tube? What is the general relation between surface tension and adhesion?

(*b*) *Internal Cohesion of Liquids.*—Note that when two glass slides are placed together with a film of water between, it requires considerable force to break the film.

Study the mechanism of spore ejection by ripe Fern sporangia. Extract water from the annulus by means of 50 per cent glycerine. The sporangium ruptures and opens; the annulus bends back; at a certain stage the cohesion of the liquid in the cells gives way suddenly with release of tension and recoil of the annulus. Observe the subsequent darker appearance of its cells due to air having replaced the water.]

6. Surface Tension and Electric Charge.—Arrange two or three dry cells in series and attach platinum wires to the terminal copper wires. Dip into a dish of dilute acid containing a drop of Hg. Which pole attracts the Hg when brought into contact with it? What is the charge on the mercury? In contact with which does the drop show greatest tendency to minimal area? Explain.

7. Negative Surface Tension. The "Myelin Forms" of Lecithin.—Place a speck of lecithin on a glass slide. Cover with a drop of water and a coverglass—preferably supported so as not to press on the object. Study the behaviour of lecithin in contact with water. How is the tendency to increase surface area reconcilable with non-mixing of the two media—which is the usual consequence of a "negative" interfacial tension? (Molecular orientation.)

II. KINETIC CONDITIONS: FORCES NOT IN EQUILIBRIUM

Movement results from upsetting the surface tension equilibrium until the balance of forces is restored.

1. Local Lowering of Surface Tension.—Place particles of camphor on clean water. Explanation of movement (given by Van

der Mensbrugghe, 1869; see Thompson, p. 212) is unequal solution of camphor and unequal lowering of surface tension on different sides of the crystal aggregates. Why does touching the surface with a greasy finger stop the movement and allow agglutination?

2. Simulation of Amoeboid Movement.—To a drop of mercury (shape and why?) lying in weak nitric acid (1N) bring a crystal of potassium dichromate? What surface tension changes are indicated by the result? Simulation of pseudopod formation, chemotaxis, etc. Note the behaviour of mercuric oxide toward the surface of the mercury. Compare following experiment (4).

3. Cleavage of a Drop by Surface Tension.—Mix olive oil with chloroform until the mixture is heavier than water. Place a drop under water in a dish and apply a small crystal of soda at each end (Spek). Explain the cleavage.

4. Tangential Movement of Surface Film.—To a little water mixed with carbon in the bottom of a petri dish present vapour of ether or drops of alcohol. Explain the vortex currents. What surface tension changes would account for the alteration in angle of contact and the recession of the water front when, for instance, ether vapour replaces air? Is the glass vapour tension increased or decreased? As a variation of the above draw a brush dipped in alcohol across a layer of red ink or eosin solution on a glass plate ("crossing the Red Sea").

5. Ingestion, Digestion, Egestion.—Present a clean glass filament and one covered with shellac (chloroform soluble) to a drop of chloroform (which may be stained with Sudan III) under water. The coated rod should be pulled in and after the shellac is dissolved may be ejected again.

Note angle of contact and adhesion with glass and shellac resp.

A more difficult but striking experiment is to draw out a fine thread of shellac (softened by heating) and allow the drop of chloroform to pull it in and curl it up inside, after the manner of the ingestion of algal filaments by protozoa.

[**6. "Secretion." Artificial Contractile Vacuoles.**—In a small drop of chloroform in water a fine mist of droplets is formed, which gradually condense to larger ones (20–30μ). When these touch the surface of the chloroform they are expelled and lost in the surrounding medium. (The droplets are probably water; they stain with water, soluble stains.) Rhumbler[1] (1898, p. 264)

[1] Arch. Entwicklungsmech. 7; 103.

describes this and other similar models, such as: (*a*) colophonium dissolved in oil of turpentine and dropped in 70 per cent alcohol, and (*b*) glycerine mixed with castor oil, a drop placed in 70 per cent alcohol.

Observe droplets of egg-yolk in water or in dilute ammonia solution. "Vacuoles" form in the yolk drops and burst to the exterior.]

Outline III

SURFACE TENSION IN CELLS

I. PROTOPLASMIC FORM

1. Figures of Minimal Area. Note the form of naked protoplasmic masses in the following: (1) the extruded protoplasm when *Sweet Pea* or other pollen grains burst in water, or a *Vaucheria* filament is cut open; (2) the plasmolyzed protoplast in short and long plant cells, such as different species of *Spirogyra*—beaded and unduloid shapes, effects of viscosity modifying surface tension; (3) vacuoles in Paramoecium and in young plant cells, as in the developing leaves at the apex of a shoot of *Elodea*.

2. Figures of Non-Minimal Area.—*Spirogyra* cells may be taken as an example. Note which surfaces within the cell tend normally to take on minimal area and which do not. Explain the shape of the nucleus (tension on threads of cytoplasm). Note particularly the irregularity of the interface between cytoplasm and vacuole and between cytoplasm and chloroplasts, also of the internal surfaces of the cytoplasm (kinoplasm).

II. PROTOPLASMIC MOVEMENT

1. Experimental Change of Form. *Apparatus.*—Dry cell and induction coil for each four students. Four bell wires with two pieces glass tubing to hold the wires which bear the induction current.

Mount filaments of *Spirogyra* lengthways on a glass slide. Pass a brief galvanic current through the filaments and observe microscopically for several minutes, regarding change in form of chloroplasts, cytoplasmic strands and nucleus. Sketch and compare with normal cells.

Consider the possible cause or causes of the changes, taking into account both surface tension and viscosity. Are the cells alive after treatment (non-staining with eosin or plasmolysis

tests)? Sketch untreated cells and stages of the subsequent transformation. The effect of chemical agents such as barium salt (M 100 or less), narcotics (chloroform), etc., and the action of gently heating one end of the slide should also be studied from the same point of view.

Other cells such as those of the staminal hairs of *Tradescantia*, *Amoeba*, etc., may be exposed to induction current and changes in the tendency to minimal area observed.

2. Amoeboid Movement; Ingestion and Excretion.—Study an amoeba in motion. The object is to find out how far the principles of surface tension as studied above appear to apply to and account for the movement. First make sure of the facts for the particular species under observation.

Observe the currents in the body of the animal. Vortex or rotary? Steady or intermittent? Is contact with a substratum necessary for streaming? (Amoebae may be suspended in weak gelatin solution.) For locomotion? In which direction does the surface film move? (Judge by adhering particles of débris or added carbon.) If possible view from the side as an amoeba travels along the edge of a coverglass. How does the angle of contact compare at anterior and posterior margins respectively?

3. Streaming Movements (Cyclosis).—In many plant cells (as already indicated in Outline I) strands of cytoplasm traversing the vacuole show active flowing movement. Suitable material: staminal hairs of *Tradescantia*; leaf cells of *Elodea*, or cells from the mesocarp of *Snowberry*. Still finer currents are often visible within the cytoplasm—good in some large species of *Spirogyra* as *S. nitida*. Consider whether local differences in surface tension might cause these movements and whether there is any evidence of such. Compare also with the myelin forms of lecithin. What are the effects of the same electrical and chemical treatment as before?

Contractile Vacuoles.—Could surface tension adequately explain the discharge of these vacuoles in *Amoeba?* In *Paramoecium?*

Ingestion.—Observe what happens when an amoeba comes in contact with algal cells or débris. If ingestion is observed, is the mechanism one of wetting or not?

Other Material.—Amoeboid cells of the blood will be studied later under the ultramicroscope. Conjugating *Spirogyra*, if available, is excellent both for movement and excretion by vacuoles.

Outline IV

ADSORPTION IN PHYSICAL SYSTEMS

I. MECHANICAL ADSORPTION

Mechanical Adsorption or concentration at a surface through the action of molecular forces is by its nature intimately related to surface tension. If a substance dissolved or dispersed in a liquid medium tends to lower the free energy at the surface it will tend to concentrate there (Gibbs' principle). At liquid-air and liquid-liquid interfaces the most important result is the formation of films and membranes, but whereas the principle of least surface can apply only to liquids, adsorption is equally or more important when one of the phases is a solid.

i. Adsorption Laws: Quantitative Experiments.

1. The Time Factor in Adsorption.—Determine the surface tension of the distilled water supplied. Measure quickly with a freshly stirred solution of bile salt 1 in 50,000 (add 0.1 cc.—or about 2 drops—of 1 in 500 to 10 cc. H_2O) and repeat at intervals (frequent at first) for 30 minutes. Disturb the liquid as little as possible. Effect of stirring? Compare protein, soap, saponin, butyric acid, etc.

2. Surface Tension and Adsorption v. Concentration of Solute.—Measure the surface tension of pure water and of solutions with increasing concentration of bile salt from say 1 in 100,000 to 1 in 500—making allowance for the time factor. Plot a curve of surface tension versus concentration. Note that as concentration increases surface tension decreases at a progressively slower rate, becoming asymptotic. Therefore, the amount adsorbed must also increase in the same way. (The latter at any concentration is actually proportional to the product of the concentration and the tangent of the slope of the above curve at that point (Gibbs' formula).)

*3. **Adsorption Isotherm.**[1]—The best adsorbent is activated charcoal (Bartell and Miller, J. Am. Chem. Soc. **44**: 1866, 1922).

Make a standard solution of oxalic acid and dilute to 0.25 N, 0.1 N, 0.05 N, 0.01 N, and 0.005 N.

Place 0.25 gram of activated charcoal in each of five steam-cleaned glass-stoppered bottles. Add to each 100 c.c. of one of the different concentrations of acid solution. Let stand for 20 minutes. Pipette off 10 c.c. of clear solution and determine the concentration of the acid by titration with 0.1 N KOH.

From these data calculate x, m, and c for each of these experiments. (x = millimoles of oxalic acid taken from the solution by the charcoal, m = grams adsorbent.)

Plot results into the form of two graphs.

(1) $\frac{x}{m}$ = ordinates, C = abscissas $\left(\frac{x}{m} = KC^{\frac{1}{n}}\right)$

(2) $\log \frac{x}{m}$ = ordinates, $\log C$ = abscissae, $\left(\log \frac{x}{m} = \log K + \frac{1}{n} \log C\right)$.

The latter should be rectilinear.

(3) Determine the constants K and $\frac{1}{n}$

ii. Examples and Effects of Adsorption: Qualitative Experiments

1. Adsorption at a Liquid-Air Interface. (*a*) *Surface Concentration.*—Take a weak (sky-blue) solution of methylene blue in a test-tube and add a little saponin to allow the making of a foam. Set aside a sample for comparison. Shake the rest for some time and separate the underlying liquid from the foam by decanting. Is there any change in the concentration of dye in the liquid (colour comparison)? Add one drop of alcohol to dispel the foam and compare also the colour of the liquid which settles out. Repeat the shaking with the decanted liquid and again compare colorimetrically. Why does the amount of foam decrease with

[1] Experiment taken from Bartell's Class Outlines of experiments in Colloid Chemistry.

repeated shakings and decantings? Why does saponin promote foaming and alcohol dispel it?

(*b*) *Concentration Membranes.*—Using a piece of glass tube as a blowpipe, blow bubbles of soap, saponin and albumin. Close the end of the tube for a minute to allow time for adsorption and then open to allow the bubble to contract under surface tension. Look for evidence of a semi-solid film on some of the bubbles as shown by shape and wrinkling of surface. Examine carefully by reflected light. Shake up a solution of albumin to a foam and after standing try to re-dissolve the froth (meringue) in water. Compare the film which forms on heated milk.

2. Adsorption at a Liquid-Liquid Interface—Emulsification. (*a*) *Emulsification.*—Shake up oil (stained with Sudan III) and water (also stained if so desired with methylene blue). Is the emulsion stable on standing? Add albumin solution to the water and repeat the process. Stability? Examine a little under the microscope for evidence of albumin films. Notice that the drops are not always spherical and may be pressed together without fusing. Move the coverglass. Any wrinkling on the surface of the droplets? This is more distinct when chloroform is used instead of oil, as water passes slowly into the chloroform bursting the membranes if the latter is internal. Ackerson, 1838, described such films as "haptogen" membranes (formed by contact) and surmised their true origin as "capillary condensation."

(*b*) "*Artificial Difflugia Shells.*"—Take chloroform mixed with finely ground glass in a narrow pointed pipette and inject drops into a watch glass of water. Notice how the glass grains come to the surface and form a shell around the drops as in Difflugia. Linseed oil and 70 per cent alcohol may be used instead as alternative liquids.

3. Distribution of Phases in Relation to Emulsifying Agent. (*a*) *Insoluble Emulsifier.*—Take equal quantities of water and benzol or xylol (stained with Sudan III) and add a very little lampblack. Shake vigorously and examine a drop of the emulsion microscopically without a coverglass. Which is the internal (droplet) phase? Which wets the particles of lampblack? Repeat with powdered $CaCO_3$ or $BaSO_4$ instead of lampblack, and compare.

(*b*) *Soluble Emulsifier—Soap in Oil-Water Emulsions* (Clowes). —Shake up equal quantities of olive oil (coloured) and an aqueous

solution of M 100 NaOH. Do the same with oil and M 100 $CaCl_2$. Examine microscopically without a coverglass. Which phase is internal in each case? While observing allow M 10 $CaCl_2$ to come in contact with the first emulsion and M 10 NaOH with the second. Reversal of phases? "A colloidal emulsifier causes the phase in which it is soluble to be the external phase" (Bancroft). Similarly a powder causes the phase which wets it to be external. Account for these laws in terms of surface tension.

4. Adsorption at a Liquid-Solid Interface: Displacement.—Shake up a solution of methylene blue with just sufficient blood charcoal to decolourize and filter. Result? Shake up a portion of the filtered charcoal with alcohol; filter and collect the filtrate. Why is dye released? (Bayliss.)

"Suspend a portion of the charcoal in approximately 100 c.c. of distilled water. Add 10–15 c.c. of benzene and shake. The dye is displaced from the charcoal and appears in the water. Shake a little of the original dye solution with benzene in a test tube. The dye is insoluble in benzene. Explain the displacement of the dye from the charcoal by the benzene. This is an example of displacement of an adsorbed substance by a liquid in which it is not soluble" (Bartell).

II. ELECTRICAL ADSORPTION

Since surfaces generally possess an electric charge (which equals the difference of potential between the two phases) oppositely charged solutes—whether ions or colloidal particles—will tend to concentrate there. This may be illustrated by experiments with stains which are generally electrolytes, either salts of a coloured organic base with an inorganic acid (hydrochloric) or of a coloured organic acid with an inorganic base (Na). The former are termed *basic* and are positively charged as to the coloured ion; the latter *acidic*, with a negative charge.

1. Staining and Electric Charge.—(*a*) Dip pieces of filter paper in equally coloured solutions methylene blue (+) and indigo carmine (−) or of basic and acid fuchsin resp. and compare rate of washing out of stain in water. What is the charge on filter paper?

(*b*) *Drop Method of Testing Stains.*—Hold a piece of filter paper horizontally and carefully place thereon a drop of each of the above and other dyes. Note difference in spreading according

to charge (an easy test for acid v. basic dyes, except those that are highly colloidal). Add successive drops in center of original stain. Follow with drops of water.

How do you account for the greater concentration round the edge of the stain with basic dyes?

2. Selective Adsorption of Ions.—One ion of an electrolyte may be adsorbed and the other remain in solution.

(*a*) Place a drop of eosinate of methylene blue (equivalent concentrations of eosin and methylene blue mixed and dialyzed) on filter paper. Which ion is held and which passes on with the water? Can you suggest how the separation of ions is possible in view of the electrostatic attraction between them?

(*b*) On filter paper place a drop of acidified (reddish) methyl orange or (yellow) bromphenol blue and of slightly alkaline (blue) bromthymol blue or (yellowish) neutral red. Note change of colour indicating a shift in the alkaline direction in the first case and in the acid direction in the second—presumably due to removal from the water (by adsorption on the filter paper) of H and OH ions respectively. Compare concentrated with dilute dye.

3. Reversal of Charge.—Adsorbed ions may neutralize or even reverse the charge on the adsorbing surface, in the latter case probably because mechanical adsorption carries the process beyond neutralization.

(*a*) Perform the drop tests with pure solutions of methylene blue and eosin. Repeat after adding a neutral salt and again after acidifying.

(*b*) Soak pieces of gelatin (1) in tap water and (2) in dilute $AlCl_3$ or $LaCl_3$. To one sample of each add a basic dye (methylene blue) and to another an acidic one (indigo carmine). Compare staining. Compare also the staining relations (electric charge) of neutral v. acidified gelatin.

Understand the terms "amphoteric substance" and "isoelectric point" in light of the above. Further examples of electrical adsorption will be found under "*Colloids*."

Outline V

ADSORPTION IN CELLS

I. MECHANICAL ADSORPTION IN CELLS

1. Boundary Films and Membranes.—Evidences of their existence, that is, visibility, prevention of mixing, semipermeability, should be examined critically in the following experiments.

(*a*) *Red Blood Cells.*—Prick finger-tip with sterile needle. Squeeze out a drop of blood on a slide and examine. Dilute with H_2O. Note swelling of red corpuscles and lacking of pigment (hemolysis). The enclosing membrane—the so-called "ghost"—persists. Treat another preparation with dilute saponin in isotonic 0.9 per cent salt solution. Result as to laking, and also shape and adhesiveness of corpuscles. How should a substance like saponin affect the membrane? Does eosin stain normal corpuscles? Heat slightly and again observe.

(*b*) *Plant Cells.*—(*Spirogyra*, onion epidermis, etc.). Record any visible indication of a differentiated film—apart from the cell wall—on the surface of the protopasm; plasmolyze the cells and observe the surface for evidence of a hyaline layer; compare *Amoeba*.

(*c*) *Fresh Surface.*—Cut across a filament of *Vaucheria* and note evidence of formation of a fresh film in some cases, in others not, on the extruded masses. Do the round globules mix with the water or stain with eosin?

(*d*) *Vacuolar Lining* (tonoplast of de Vries). Plasmolyze *Spirogyra* with normal KNO_3 and eosin (a trace of iodine may be included). Note that the bulk of the protoplasm may be killed (stained) in some cells while the boundary film of the vacuole plasmolyzes and bars the diffusion of eosin. Red onion cells may be used without having dye in the external medium. When strongly plasmolyzed, irrigate with water; the vacuolar membrane may expand and burst through the stiffened protoplast.

(*e*) *Nuclear and Plastic Membranes.*—Crush filaments of *Spirogyra* under a coverglass and observe that in the resulting swelling and successive coagulation the nucleus and chloroplasts may become separated from their membranes, which are then more clearly revealed. Has the nucleus one or two envelopes?

2. Effect of Capillary Active Substances on Protoplasmic Membranes. (*a*) *Traube's Rule.*—If the concentration of substance at protoplasmic boundaries is in any measure due to adsorption, the presence of another substance of high capillary activity ought to effect substitution and a change in the character of the membrane. Kamm (Science, 1921) says that the toxicity of the alcohols is proportional to their surface activity, which increases three times in each successive number of the series, ethyl alcohol being taken as unity (Traube's rule). Taking 11 per cent ethyl alcohol as the " critical concentration " for rapid toxic action on *Spirogyra* (Czapek) the critical concentration of butyl alcohol ought accordingly to be

$$\frac{11}{3 \times 3} = \text{approximately } 1\tfrac{1}{4} \text{ per cent}$$

Try if 11 per cent alcohol (ethyl) and $1\frac{1}{4}$ per cent butyl alcohol have the same surface tension and the same degree of toxicity (time taken to kill the cells). Also test other alcohols, acetones, etc. Does it follow, however, that equal lowering of surface tension at the water-air interface implies equal lowering at the surface of the cell?

(*b*) Recall laking of blood cells by saponin and other capillary active agents.

3. Influence of Adsorption on Chemical Processes.—

(1) *Inactivation resulting.*

In vitro.

(*a*) Add 1 part $K_4Fe(CN)_6$ about M/900 to 8 parts water. Then add 1 part $FeCl_3$ M 100. Immediate prussian blue reaction. Repeat using aluminium hydroxide solution instead of water. Does the reaction take place? Explain in terms of adsorption, remembering that Al_2O_3 particles are positively charged.

(*b*) To two test-tubes of warm milk add a little rennet solution, in one case first shaking up the rennet with carbon. Difference in enzyme activity?

(*c*) Why does milk remove the astringency of strong tea?

In vivo.

Note the astringency of various unripe fruits, like green bananas, and absence of astringency in the ripened fruit. Treat pulp of each with $FeCl_3$ and examine under the microscope. Blue colouration of tannin cells is equal after a time in ripe and unripe. Does the reaction result in a precipitate in the ripe fruit? Compare in this respect tannic acid and $FeCl_3$ reacting in solution. Examine microscopically. The tannin is adsorbed by a cellulose-like body in the cells (Lloyd 1912).

Apply alkaloid (caffeine 1 per cent) to ripe and unripe. Precipitate only in latter. To other preparations apply weak alkali. In unripe banana there is swelling of the colloid complex; tannin diffuses out and forms precipitation membrane. In ripe banana it does not diffuse out.

(2) *Activation resulting* (Experimentation proper to biochemistry).

Enzyme action like inorganic catalysis is partly brought about by adsorption of the reagents (see Bayliss). Oxidation of foodstuff (amino acids) takes place at ordinary temperatures when these are adsorbed on charcoal containing a trace of iron (Warburg). Similarly chemical processes which go on in cells, but not in test-tubes, are ascribed largely to adsorption and allied phenomena.

II. ELECTRICAL ADSORPTION IN CELLS

1. Post Mortem Staining. (*a*) *Staining and H-ion Concentration.*—Use cells of *Spirogyra*, onion, etc., which have been killed by dipping in alcohol. Stain with methylene blue and methyl orange respectively, (*a*) in an alkaline and (*b*) in an acid medium. Compare any other basic versus acidic stain.

(*b*) *Double Staining.*—Stain similarly treated cells with a basic dye followed by an acidic one—best in a slightly acidified medium. What parts of the cell have an affinity for the respective dyes? Inference as to isoelectric point? Treat a section of a plant *stem* in the same way, comparing the various tissues as regards adsorption of basic versus acid dye.

2. Vital Staining. *Basic Dye.*—Allow living *Spirogyra* or other cells to become stained with methylene blue or neutral red. Does

the stain wash out readily in water? In M 20 $CaCl_2$? Why does the cell wall lose colour in $CaCl_2$? Does it stain more strongly with *acidic dye* in presence of $CaCl_2$ than in H_2O alone?

Staining of the Cell Interior.—Mount *Spirogyra* in a solution of methylene blue or neutral red plus $CaCl_2$ M 20 (this prevents staining of the cell wall but does not affect the interior, since it fails to enter the cell). Do the particles which stain in the cytoplasm pre-exist as granules or are they a precipitation product? Why do they tend to flocculate and coalesce on staining (surface tension versus electric charge)? Does the vacuole stain uniformly at first? Does a precipitate form later? Nature of this precipitate?—solid or liquid? What satins and what does not stain in the living cell? Compare with post mortem staining. Irrigate with 1 per cent caffeine and observe behaviour of stained substance. Treat stained cells with acetic acid M 1000. Does the stain tend to be released?

Acidic Dyes.—Some cells (in tissues of many plants) are freely permeable to acidic as well as basic dyes. Allow cut leaves (as of Anthericum) to take up acid fuchsin or immerse sections in it and after a time observe the distribution of stain in the cells.

Note that acidic dyes (eosin, acid fuchsin, etc.) do not normally enter cells of *Spirogyra* in life. To effect entry plasmolyze strongly with sugar and, thereafter, deplasmolyze suddenly with dye solution. The plasma membrane during the extension becomes temporarily more permeable especially in the case of a viscid protoplast (*Spirogyra maxima* is suitable) and some cells at least will be found to have been penetrated by dye). What stains? Wash in water. Is there adsorption?

The amphoteric nature of the substance which ordinarily adsorbs a basic dye may be shown thus. First treat filaments with 1 per cent caffeine in order to cause the stainable material to run into large drops. Then apply rather strong acidic stain in N/1000 acetic acid. The acid tends to disperse the precipitate but before the large drops dissolve, they may be seen to stain deeply.

Other Material.—Experiment with vital staining of animal cells, for instance, Amoeba, ova (in oviduct) of cockroach (in frog's saline, half strength), tissues of the frog (in Frog's saline—0.7 per cent NaCl), etc.

Outline VI

DIFFUSION AND OSMOSIS IN PHYSICAL SYSTEMS

1. Factors Influencing Rate of Diffusion. (1) *Gradient of Concentration.*—Fill four test tubes with hot $1\frac{1}{2}$ per cent agar containing a suitable indicator (brom cresol green or methyl red). When solidified place open end down in bottles of H_2SO_4 of different concentration, say 0.5 M, and 0.1 M. (Set up the next experiment with the other tubes.) Measure by the change in colour of the indicator the approximate rate of progression $\left(\frac{\text{distance}}{\text{time}}\right)$ in each tube during the laboratory period, also the average rate for successive days. How does the rate compare in the respective tubes and how does it vary with lapse of time? Get a clear idea of *gradient of concentration* and of the relation to it of rate of diffusion.

Rate of diffusion is the amount of substance passing a given point in a given time $\left(\frac{s}{t}\right)$ and is not necessarily proportional to the rate of advance of the diffusion front except for identical concentrations at the start, as in the following experiments.

(2) *Size of the Diffusing Particles.* (*a*) Simultaneously with the above set up similar experiments with equal concentrations (0.5 M) of HCl and citric acid. Compare rate of diffusion (preferably from time taken to travel equal distances) and relate to molecular weight.

(*b*) Partly fill two vials with warm agar mixed with neutral red. When this has set add N 10 KOH and N/10 NaOH. Cork and set aside. Consider ionic mobility in relation to hydration of ions.

(*c*) Alternatively, dyes may be used, but the size of the diffusing particles is uncertain apart from the result of diffusion experiments.

Partly fill two or more vials with warm gelatin or agar. When it has set pour in eosin, Congo red, etc., of approximately the same percentage concentration. Congo red is highly colloidal.

(3) *The Viscosity of the Medium*—frictional resistance to diffusion. Compare the rate of diffusion of the same eosin solution as above in a more concentrated gelatin gel.

(4) *Diffusion Aided by Mass Mixing.*—Drops of an aqueous solution of methylene blue are placed on the surface of water in a dish. Compare drops of an alcoholic solution. Why does the latter disperse so rapidly? True diffusion? Consider surface tension between alcohol and water.

2. Semipermeable Membranes: Osmotic Phenomena.—Three vials are quarter-filled with warm agar or gelatin containing 2 per cent $K_4Fe(CN)_6$. After this has set, the sol alone, as cool as it will run, is poured into each up to two-thirds of the tube, and, again on setting, $CuSO_4$ solution is added in three different concentrations, one 5 per cent (isotonic with the 2 per cent $K_4Fe(CN)_6$), another weaker, say 1 per cent, and the third stronger, say 10 per cent. Cork and set aside. A U-tube with gel in the lower part and aqueous solution in each arm is a better variation of the above (Pringsheim, Jahr. f. w. Bot. **28:** 1, 1895).

From the position of the precipitation membrane next day determine the relative rate of diffusion of the two salts and of different concentrations of the same salt ($CuSO_4$). Explain curvature of membrane in some of the tubes. Does the membrane change position? (Mark position and continue the experiment.) If so, why? Why does it become thicker in some of the tubes? Is the membrane permeable to the salts? To water?

Understand permeability, impermeability, semipermeability, osmosis, osmotic pressure, tonicity, hyper-, iso- and hypo-tonicity as illustrated in the above experiment.

3. Physical "Models" of Living Growth.—(*a*) Drop a crystal of $K_4Fe(CN)_6$ into a 3 per cent solution of $CuSO_4$ or better suspend the crystal by a thread. Leave undisturbed and observe the character and mechanism of the resulting growths.

(*b*) Reverse conditions, placing a crystal of $CuSO_4$ in a solution of $K_4Fe(CN)_6$.

(*c*) Drop small crystals of $CoCl_2$ into a weak solution of sodium silicate. Vary the concentration of the latter.

Further examples to be studied if desired:

(*d*) A lump of fused $CaCl_2$ in the bottom of a tall jar of concentrated Na_2CO_3 is set aside for several days.

(*e*) A drop of 2 per cent tannic acid containing sugar (say N/2) is introduced by a fine pipette into the interior of a gelatin solution. What part does the sugar play in the result?

References.—Traube, 1867; Walden, 1892 (see Bayliss); Leduc, Théorie physico-chemique de la vie, 1910.

[**4. Comparison of Osmotic Pressures by Use of a Semipermeable Membrane.**—(Since these experiments are slow and are duplicated in principle by the plasmolytic ones which follow they may be omitted if time demands.)

Construction of Ferrocyanide Membrane (Tammann's method; see Philip's Physical Chemistry). Dip the end of a short glass tube about 1 cc. in diameter into melted gelatin (say 20 per cent) to which a little $K_2Cr_2O_7$ has been added. Suspend, film end downward, until the gelatin stiffens. Allow to dry in light. This treatment renders gelatin insoluble. The dichromate should be washed out by soaking in water.

Put some $K_4Fe(CN)_6$ (2.1 grams per liter) inside the tube and suspend in vessel with $CuSO_4$ (2.5 grams per liter) until a brown $Cu_2Fe(CN)_6$ precipitate covers the whole gelatin membrane. Do not allow the membrane to dry after this.

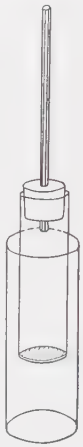

Fig. 61.

Use.—Fill the tube with molar cane sugar. Cork with a rubber stopper holding a capillary tube, inserting gently to avoid rupturing the membrane. It is well to have in the sugar solution $K_4Fe(CN)_6$ (say 0.31 gram per liter) and to have isotonic $CuSO_4$ (0.84 gram per liter) outside; this will repair any break in the $Cu_2Fe(CN)_6$ membrane. Otherwise only H_2O is outside.

Adjust the stopper so that meniscus is just above it. Mark the position when the tube is set in water (or water and dilute $CuSO_4$) and measure the distance to which the liquid rises in say half an hour.

Repeat as above with an unknown solution X. Since osmosis varies as the difference in osmotic pressure on the respective sides of the membrane,

$$\frac{\text{Rate 2}}{\text{Rate 1}} = \frac{\text{Osmotic pressure of } X}{\text{Osmotic pressure of molar cane sugar}}$$

Regarding X as a non-electrolyte like cane sugar its molar concentration will therefore be $= \frac{\text{Rate 2}}{\text{Rate 1}}$

Having calculated it, make up the same concentration of cane sugar and balance against the unknown in the osmometer as before.

The rate of end- or exosmosis as compared with the Rate 2 will give data for a second approximation. If desired a third approximation can be made in which great accuracy is possible.

Determination of osmotic pressure of electrolytes, their isotonic coefficients, etc., which might be made by the above method are given under Plasmolytic Methods.]

5. Permeability of Membranes.—(1) *Penetration by Pores.*—(*a*) Using a membrane of $Cu_2Fe(CN)_6$ and an osmometer as in previous exercise, note that the membrane is not impermeable to salts of low molecular volume; for example, test with $AgNO_3$ for the passage of chloride after some time has elapsed.

(*b*) Membranes of wider mesh such as parchment paper or dead plant or animal membranes (for instance, tanned pig's gut) will differentiate between larger molecules, that is, sugar or methylene blue as opposed to aniline blue. Tie the membrane tightly over the end of an osmometer tube and allow time for diffusion.

(2) *Solution in the "Membrane"*—(*a*) Demonstration: Shake up phenol and water in a test tube to separate into two mutually saturated layers—the aqueous phase is the lighter. With a pipette carefully run a layer of water saturated with $CuSO_4$ as well as phenol, into the bottom of the tube. Also gently stir a drop of neutral red or methyl red into the upper (aqueous) layer. Mark position of boundaries. Cork or pour oil on top. Set aside and do not disturb for a few days or even weeks. Observe diffusion of the coloured solutes and slow aqueous osmosis. To what extent does the phenol layer act as a permeable and to what extent as a semipermeable membrane?

(*b*) "Lipoid Solubility."—Shake up a xylol solution of lecithin with an aqueous solution of neutral red and mount drops on a glass slide. Does the xylol-lecithin take up the dye? To the edge of a drop of emulsion on a slide without a coverslip apply a small drop

of NH_4OH (N 100 or weaker) and to another apply NaOH of the same or weaker concentration. Which enters the drops and why? [Repeat with a coverglass and observe amoeboid movement and vortex currents of drops as alkali reaches them—surface tension effects.] Consider the analogies in this experiment with cell permeability (see later).

3. *Penetration by Adsorption.*—Demonstration: Shake up animal charcoal with methylene blue solution and filter. Emulsify the carbon paste with oil (nujol). Set up a test tube with (1) *phenol* in the bottom, then (2) *water* and (3) on top the above *emulsion.* Carbon particles gradually detach themselves and fall through the water (without visibly colouring it) to the phenol, which soon becomes deep blue.

Outline VII

DIFFUSION AND OSMOSIS IN CELLS

1. Osmotic Pressure in Living Cells. (1) *Plant Cells.*—For best results use material in which (1) the walls are relatively rigid, (2) the protoplast is not adhesive to the cell wall, and (3) the inner layers of the wall do not thicken when internal pressure is released—as it does, as in some *Spirogyra* samples. A coloured sap vacuole also facilitates observation. The coloured epidermis of certain leaves such as Coleus, and filaments of some kinds of *Spirogyra* satisfy the requirements.

Note that a strong solution of cane sugar (M/2) causes the protoplasm to contract away from the wall (=*plasmolysis*). Distinguish the protoplasmic layer from the vacuole. Addition of sufficient water causes restitution (=deplasmolysis).

Set up *Coleus* epidermis or *Spirogyra* in a series of concentrations of cane sugar (say M/2, M/3, M/4, M/5) in shallow dishes and after a few minutes observe carefully under the microscope. Which concentration is most nearly *isotonic* with the average cell—just plasmolyzes 50 per cent of freely exposed cells?

(*Note.*—The outer surface of epidermal cells is cuticularized, therefore study cells whose inner surface is not covered by tissue).

Repeat with a closer series of concentrations around isotonic so as to determine the isotonic concentration more exactly.

Express the *osmotic value* of the cells at incipient plasmolysis in terms of the molar concentration of cane sugar, which is isotonic. Deduce the osmotic pressure of an average cell, starting with the fact that the osmotic pressure of

molar cane sugar = 22.4 atm.

(2) *Animal Cells. Erythrocytes.*—Recall or repeat the hemolysis of blood cells by water. Note also their shrinkage and wrinkling of the membrane (crenation) in M/1 NaCl, and their unchanged volume in 0.9 per cent NaCl.

Muscle.—Weigh a frog's gastrocnemius before and after a prolonged immersion in hypotonic salt solution, isotonic (0.7 p. c. NaCl), and water.

Other Animal Material.—Protozoan parasites such as ciliates from the frog's intestine and a gregarine from the cricket may be tested for volume change in relation to osmotic pressure of medium.

2. Plasmolytic Methods of Comparing Osmotic Pressures.—(1) An unknown solution *X* able to plasmolyze and non-toxic is supplied. Find its osmotic pressure by either of the following plasmolytic methods.

(*a*) *Plasmometric Method.*—(A micrometer eyepiece is required.) If suitable cells of cylindrical shape (as firm-walled species of *Spirogyra*) are available, determine the volume of the plasmolyzed protoplast in the unknown and in a $CaCl_2$ or cane sugar solution of known osmotic pressure (volume $= l - \frac{1}{3} d$, see p. 104). The osmotic pressures are inversely proportional to the volumes.

(*b*) *De Vries' Incipient Plasmolysis Method.*—Using cells whose osmotic value has already been determined by the plasmolytic method, place a series of specimens in various dilutions of the unknown. Allow ten minutes and observe which is most nearly isotonic. This may be followed by a series of narrower range to discover more accurately the isotonic fraction. By proportion the osmotic pressure of the original undiluted solution is simply calculated if the degree of dilution and the osmotic pressure of the undiluted solution are known.

(2) *Isotonic Coefficients.*—Make up solutions of NaCl and of $CaCl_2$ approximately isotonic with M/3 cane sugar (*i* for NaCl = *ca.* 1.7, for $CaCl_2$ = *ca.* 2.3). Test plasmometrically, using *Coleus* epidermis. Is the degree of plasmolysis the same? Test also with osmometer. If possible determine *i* for $CaCl_2$ with greater precision. Calculate the apparent dissociation of the two salts from the formula $i = 1 + (n - 1)\ \alpha$, where n is the number of ions from one molecule and α is the dissociated fraction.

3. Rate of Diffusion into Living Cells.—Methods of measuring cell "permeability."

(1) *Plasmolytic Method.*—Place epidermis of Coleus (or leaves of *Elodea* or other material) in M/2 and M/3 of each of the following: Cane sugar, glucose, glycerol, urea, alcohol. Do all plasmolyze? Is plasmolysis (when it appears) permanent (at least

until the end of the laboratory period) or of limited duration? Judging by deplasmolysis state which penetrate and in what order of speed.

(2) *Precipitate within the Cells.*—Compare the speed with which 1 per cent caffeine and 1 per cent urea (=about M/16) produce a precipitate with the tannin in the sap of *Spirogyra*. Does the rate depend upon molecular size? Mol. wt. caffeine 212, urea 60.

(3) *Reaction with Indicators. Penetration of Acids and Alkalis.*—Plant cells which contain natural indicators may be used or others may be allowed first to take up an indicator (neutral red).

Weak v. Strong Base.—Treat Coleus epidermis (or *Elodea* stained with neutral red) with NH_4OH(M 200) and NaOH (both M/200 and M/2000). In which do the cells become blue? Does the red colour return in tap water? Are the cells still alive? Does NaOH penetrate during life? Transfer cells blue with NH_3 to N/2000 NaOH. Explain the result.

Weak v. Strong Acid.—Compare the rate of colour change in the petals of *Symphytum*, or of dispersion of the precipitate produced by caffeine in *Spirogyra* when acetic acid (M 1000) and HCl (M/10,000) resp. are applied. (The *p*H is about the same.)

(4) *Penetration of Stains.*—Acidic v. basic dyes have already been compared. What is the general conclusion?

(5) *Relation to Size of Particle.*—Using tissues which are readily permeable to acid dyes, such as leaves of *Vicia* or *Anthericum*, etc., expose sections, several in each dish, to 1 per cent orange G. eosin and congo red. At intervals mount a section in water and quickly note if cell sap is coloured. Order of rate of penetration? Does congo red penetrate the cell wall? Compare with rate of diffusion in gelatin (above) also with rate of capillary diffusion when drops are placed on filter paper, that is, rate dye travels relative to water. (Ruhland, Jahr. f. wiss. Bot. 51.)

4. Modification of Permeability. (1) *Death Changes.*—The fact that death of the cell increases its permeability for many substances which normally enter with difficulty should already be familiar. Take, for example, penetration of eosin or capacity for plasmolysis in *Spirogyra* after treatment with iodine, or escape of pigment from slices of beet before and after heating.

(2) *Effects of Narcotics.*—Compare the rate of staining of *Spirogyra* by very dilute methylene blue (*a*) in water, (*b*) in 1 per

cent ether, (*c*) in 5 per cent ether. Does 5 per cent ether produce irreversible injury? Does 1 per cent.

(3) *Effects of Ions—"Antagonism."*—(*a*) Place filaments of *Spirogyra* or epidermis of *Coleus* in (*a*) 0.28 M NaCl, (*b*) 0.2 M $CaCl_2$ (approximately isotonic with (*a*)), and (*c*) a mixture of the two with NaCl predominating. Observe at intervals for an hour or longer. Does plasmolysis persist, or if not in which does deplasmolysis supervene? Consider penetration of Na as compared with Ca, and of Na in absence and presence of Ca. Effect of Ca on permeability.

(*b*) Make one preparation of pure M 100 $BaCl_2$, another of M/100 $BaCl_2$ 9 parts, M 2 $CaCl_2$ 1 part. Place filaments of *Spirogyra* in each and compare the penetration of Ba, using changes in the chloroplasts as criterion. Antagonism.

(*c*) Compare the "antagonistic" action of the polyvalent cations. To separate 10 cc. quanta of M 100 $BaCl_2$ add a drop of (1) M 10 $CaCl_2$; (2) M 10 $SrCl_2$ or $ZnCl_2$ and (3) M/10 YCl_3 or $AlCl_3$. Compare penetration of Ba in each and in pure water. Relation of antagonistic action to valency of cations? Also compare the rate of penetration of dilute Methylene Blue into *Spirogyra* cells in H_2O, M/20 $CaCl_2$ and M/100 $LaCl_3$.

(*d*) Rate of penetration in relation to concentration. Compare the relative times taken for M/10, M/100 and M/1000 $BaCl_2$ to produce contraction of chloroplasts. Is the rate proportional to concentration gradient. Compare times taken for NH_4OH M/100, M/500 and M/2500 to turn Coleus epidermis blue? Is this proportional to gradient of concentration? Does $CaCl_2$ have any effect on rate of penetration of ammonia?

5. Turgidity and Movement.—Measure thin strips from the internal tissue of rhubarb or celery stalk, and also strips including epidermis on one side, and lay for some time in a series of solutions of salt or sugar ranging from marked hypo- to marked hypertonicity. Explain resulting differences in rigidity, length and curvature. Kill strips by heating and treat in same way. Any response?

Epidermis of a leaf of *Tradescantia* is immersed in water with a trace of ammonia added and the form of the guard cells and stomata noted and sketched. (This depends partly on whether the adjoining cells are alive or dead. Why?) Treat with M/10 $CaCl_2$ and compare. Explain closure of the stomata.

Outline VIII

H-ION DETERMINATION IN PHYSICAL SYSTEMS AND IN CELLS

1. Indicator Method. *Equipment.*—Standard solutions, viz.: sets of buffer solutions with indicator. Sets sold in sealed ampoules, as by LaMotte, are handy. (For preparation of standard solutions see Clark or Cole.) Test tubes of the same bore as the standard. Clean carefully each time. Indicator solutions in bottles with dropping pipette. (Hold vertically and deliver slowly.)

Method.—Use 1 drop of indicator (0.04 per cent) to 1 or 2 cc. of fluid to be tested. The concentration must be the same as in the standard. 10 cc. (or 5 cc.) is the common amount of solution for a test.

If the *p*H is approximately known, use an indicator of corresponding range. If it is quite unknown, one may begin with a " universal indicator " (B.D.H.) or some such mixture; or if we start with some intermediate indicator, the colour obtained tells in which direction to proceed to find an indicator whose range includes the *p*H of the unknown. When this is reached, hold tube and standard side by side to white light. The tint of the unknown must either correspond to a standard or be intermediate between two successive ones.

Better comparison is possible with the aid of a *comparator*, Fig. 62, especially if the unknown is coloured or turbid. A comparator may be made by drilling holes in a block of wood as illustrated. Three in a row are used for clear solutions, the unknown in the middle and standards on either side. With coloured solutions the back row enables one to look through the same depth of colour in each case as illustrated in Fig. 62 and Fig. 64.

The most accurate work demands a manufactured colorimeter.

***2. Potentiometric Method.**—Special apparatus: Galvanometer, Wheatstone bridge, two dry cells, standard Weston cell;

potentiometer, two bright platinum (or gold) electrodes, two vials, KCl bridge, standard acetate solution; quinhydrone crystals; balance; burette and pipettes for titration.

To understand the principle of the potentiometer it is a good plan for the student to use first an ordinary meter bridge with a battery of two dry cells in series and a galvanometer as described in the theoretical part, p. 120. To calibrate the wire of the bridge one may use a standard cell in the position which the concentration cell (x) usually occupies (Fig. 63). The sliding contact L will then occupy another position, say L_1, when the

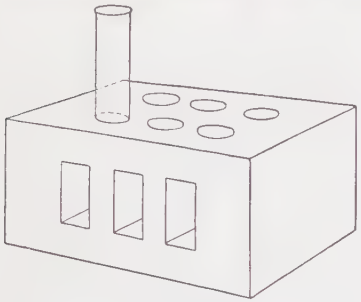

Fig. 62.

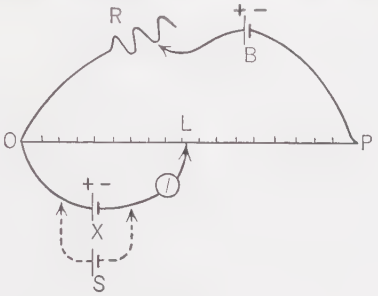

Fig. 63.

two E.M.F.'s. balance as shown by the nil reading of the galvanometer. Then

$$\frac{OL}{OL_1} = \frac{\text{E.M.F. of concentration cell}}{\text{E.M.F. of standard cell.}}$$

In actual practice it is much more convenient to use a potentiometer instead of a wire with a sliding contact.

Apparatus for use with double Quinhydrone electrode:

2 bright platinum or gold electrodes, with tubes for mercury.
2 vials.
Saturated KCl bridge.
Standard acetate of known pH.
Quinhydrone crystals.

The KCl bridge may conveniently be a narrow tube bent into U shape and filled with agar containing saturated KCl, or it may even be a piece of string saturated with KCl. When not in use dip ends into saturated KCl.

Procedure, in brief, when double quinhydrone electrode is used:

(*a*) *Standardize potentiometer* with standard cell; that is, adjust rheostat so that E.M.F. of standard cell is recorded correctly. *It is important that taps on key while observing deflection be always only momentary.*

(*b*) *Set up concentration cell* thus: —One electrode vessel should contain standard acetate, the other the solution to be tested (say 10 cc.). Stir into each a knife point of quinhydrone. Connect by KCl bridge; immerse electrodes and insert the wires from the potentiometer into the tubes containing mercury at heads of electrodes.

(*c*) *Measure E.M.F. of Concentration Cell.*—Set dials of potentiometer to zero. Tap key. If galvanometer deflection is to left change over the wires at the electrodes so as to get + to +. When deflection is to right turn first coarse and then fine adjusting dials so as to increase reading till the galvanometer registers no current. The dials then record the E.M.F. of the concentration cell.

(*d*) *Calculate* pH as follows: If standard acetate is + (connected with + terminal of instrument) $p\text{H} = 4.63 + \frac{\text{E}}{F}$. If test solution is $p\text{H} = 4.63 - \frac{\text{E}}{F}$

E is the observed E.M.F. (millivolts).

F is 57.7 at 18° C., + .02 for each degree (see theoretical part).

3. pH Change in Titration. (1) *Titration Curve of Acetic Acid.* *(*a*) *Potentiometeric Determination of* pH.—Prepare 0.2 N NaOH. Measure 20 cc. acid into clear beaker or vial and measure pH by quinhydrone method. Titrate with soda by 2 cc. stages and take pH at each stage. (At which stage does the chain give zero E.M.F.?) Plot curve of pH against cubic centimeters added alkali. Calculate the approximate theoretical pH at each stage from formula (p. 111):

$$p\text{H} = \log \frac{1}{\text{K}_a} + \log \frac{[\text{salt}]}{[\text{acid}]}$$

$$\log \frac{1}{\text{K}_a} \text{ for acetic acid} = 4.74$$

Plot theoretical curve and compare with observed curve.

(*b*) *p*H *Determination by Means of Indicators.*—This may be done in three different titrations with an appropriate indicator for each stage of the titration added to the solution in the vial.

*(2) *Comparison of Results Obtained by Electrical and Colorimetric Methods.*—(*a*) 0.2 N acetic acid half neutralized by NaOH (0.2 N) (or acetic acid and Na acetate in equal concentration 0.2N). Indicator methyl red.

(*b*) Acid potassium phosphate 0.2 M
Basic sodium phosphate 0.2 M

Mix in proportion of 4 to 6. Indicator brom thymol blue. Estimate the *p*H of each of above as carefully as you can by indicator method and repeat with potentiometer method.

4. Buffer Action. (1) *Resistance to Effect of Acid and Alkali on p*H.—To 10 cc. each of boiled distilled water, boiled KCl solution, and above phosphate mixture, add neutral red or brom thymol blue. The colours of the various media will not differ greatly, all being near the neutral point.

To other 10 cc. portions add 5 cc. brom cresol green or other indicator with acid range. Titrate the phosphate solution with 0.2 N HCl until brom cresol green becomes green and compare the effect of the merest trace of acid on the other solutions.

(2) *Effect of Dilution.*—(*a*) Dilute phosphate and standard acetate buffers ten times. Test 10 cc. of original and diluted solutions with appropriate indicators—brom thymol blue, brom cresol green, or methyl red.

(*b*) To 10 cc. of original and diluted standard acetate add 5 cc. phenol phthalein. Titrate each with 0.2 N NaOH till faint pink appears. Compare amounts of alkali required.

5. Sources of Error in Biological Use of Indicators. (*a*) *Reaction with the Indicator.*—To a dilute and to a concentrated solution resp. of brom phenol blue add enough very dilute HCl to change colour. Compare the amounts required. (As already seen, dyes often accumulate in much higher concentration in cell sap than in the bathing solution.)

(*b*) *Adsorption of Indicator.*—Allow pieces of gelatin to lie for some hours in dilute brom phenol blue. Does the gelatin adsorb the stain? Add just enough acid to turn the aqueous solution yellow. Does the gelatin also change colour? Try the effect of further addition of acid. (Dyes which accumulate in cells frequently do so by adsorption.) The $[H^{\cdot}]$ within the gelatin is

actually lower than outside (Donnan equilibrium) but the indicator colour is no criterion of the difference since adsorbed indicator is not free to respond.

(*c*) *The internal pH of cells does not necessarily vary as the external pH.* (Differential permeability.)

Expose cells, as of *Elodea*, which have taken up neutral red to NH_4Cl M 10. What reaction is produced within the cell and what is the reaction of the NH_4Cl solution itself? Explain. Expose the same to NH_4OH N 1000 till neutral red becomes yellow. Transfer to NaOH N/2000. The cells change to red. Why?

6. Some Applications to Fluids. *Determine*: (1) *p*H of coloured fluid, for example, *urine*, by the indicator method. Dilute three times to reduce colour. Place 5 cc. in 3 test-tubes to occupy holes 1, 4 and 5 as illustrated. Find by rough test the indicator in whose range the sample lies. The tube to occupy hole 4 receives the indicator, 3 holds water, 2 and 6 colour standards.

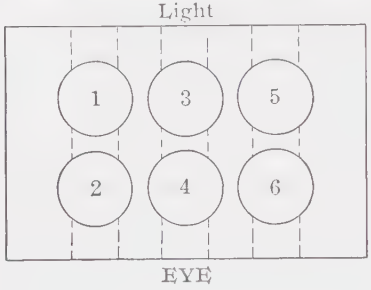

Fig. 64.

(2) *p*H of turbid solution, such as *soil extracts* (soil extract is usually well enough buffered to stand dilution when indicator method is used). Use comparator as above.

(3) *p*H of culture media at intervals during growth, for example, *plant water cultures, yeast cultures, lactic acid fermentation.*

7. Some Applications to Cells.—(1) Observe colour changes in food vacuoles of *feeding Paramoecium* when medium contains dilute neutral red, methyl red, pigment from red cabbage, or other appropriate indicator.

(2) Allow tangential section from under side of *Tradescantia* leaves to take up methyl red in darkness until the guard cells are pink. Expose to bright light and observe apparent loss of colour—really due to change to pale yellow (add acetic acid M 2000 and red returns). Why does light make the reaction more alkaline? (CO_2 is used in photosynthesis. Guard cells contain chlorophyll.)

Leaves or portions of leaves may be infiltrated with a solution of indicator by centrifuging. Allow stomatal chambers of leaves

which have been kept in the dark to become filled with brom cresol purple. Effect of exposure to red, green and blue light resp. ? Reduction in H-ion concentration indicates photosynthetic assimilation of CO_2 and consequent reduction of CO_2 tension in intercellular spaces.

Outline IX*

BIOELECTRIC CURRENTS AND BIOELECTRIC POTENTIAL

Special equipment: 2 capillary electrometers; 2 calomel electrodes; dry cells; pig's bladder, open tubes, clay.

Measurment of potential in tissues can be done in the same way as measurement of the potential of a concentration cell, namely, by means of the Wheatstone bridge or potentiometer. The ordinary potentiometer contains a galvanometer as detector of current flow. Actively functioning plant and animal tissues produce changes of potential which are so rapid that the inertia of the coil in a d'Arsonval galvanometer renders it less suitable for the detection than a capillary electrometer.

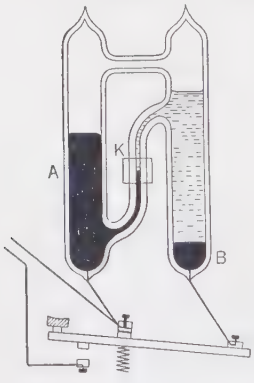

Fig. 65.—(After Clark.)

The capillary electrometer is extensively used in animal physiology. Description of the various types of instrument and their use may be found in the textbooks. The accompanying diagram (from Clark) illustrates one type. The capillary electrometer depends for its action upon the alteration of (apparent) surface tension between mercury and H_2SO_4 accompanying alteration of the potential at the surface. Reduction of potential causes increase of tension of the meniscus in the capillary tube which forces the mercury back until the weight of the other column prevents further retreat. If the wires from the respective Hg masses are at the same potential there is no movement. A horizontal microscope is focused on the meniscus, which is rendered clearer by cementing a coverglass to the capillary with Canada balsam. The electrometer is short circuited when not in use. The key makes the short circuit when released.

When pressed down it breaks it and brings the electrometer into the circuit to be observed.

In all cases of measurement of potential and observation of bioelectric currents non-polarizable electrodes should be used to connect the tissue with the instrument. In the following experiments two calomel electrodes are recommended for this purpose.

Experiments with Apple

1*. Concentration Effect.—(*a*) Place an uninjured apple in a shallow dish of M 10 NaCl. In the hollow on the top of the apple place a drop of the same liquid. Calomel electrodes (sat. KCl) connected with a potentiometer are dipped into each. If all is correct there should be no potential difference recorded. Change one of the solutions to a different concentration and repeat.

(*b*) Bring two uninjured portions of an apple as before in connection with solutions of different concentration, varying the concentration while keeping the ratio the same thus:

N/10 and N/50, N/50 and N/250, N/250 and N/1250.

How does potential difference vary with concentration? What is the significance as regards relative permeability of Na and Cl into apple skin?

2*. Ion Effect.—Measure the potential difference when KCl, versus NaCl and KCl versus $MgCl_2$ of the same molar concentration are used. Test also K_2SO_4 versus KNO_3.

3*. Potential Difference between Uninjured and Injured Region (Current of Injury).—Injure the skin of the apple at some point and bring the same solution, say M/10 KCl, separately in contact with the uninjured and injured surfaces respectively. Which is more negative, injured or uninjured? Compare slight injury with a deep cut.

4*. Potential Difference between Different Organs.—Is there any potential difference between stem and leaf of a growing plant?

Experiments with Frog—(From E. N. Harvey's "Laboratory Directions in General Physiology"). For most of these experiments a capillary electrometer is desirable.

5*. The Action Current of the Heart.—Non-polarizable electrodes are applied to ventricle and auricle of the frog's heart while in the body and connected to the capillary electrometer on the

microscope. Heart muscle, like every other muscle, becomes during contraction electrically negative relatively to inactive portions of the tissue. A wave of contraction accompanied by a wave of negative potential passes over the heart and is recorded by the electrometer. Do the excursions of the mercury correspond to heart beats?

6*. The Current of Injury (Current of Rest or Demarcation Current) of Muscle.—A sartorius muscle is carefully prepared and one end cut off. Non-polarizable electrodes are placed one on the *un*injured surface, near one end, and the other on the *un*injured surface, near the other end, and led off to the capillary electrometer. A deflection may be noted on opening the short circuiting key, indicating differences in the electrodes. Note its direction and mount. Now one of the non-polarizable electrodes is placed on the *injured* end, the other is left near the uninjured end of the muscle. Again note the deflection of the electrometer. Is it greater than before? In which direction does it indicate that a current is flowing?

7*. Action Current and Negative Variation.—Stimulate the muscle by pinching while a current of injury is flowing. How is the electrometer affected? Do you see now why the action current was called the "negative variation" of the current of rest?

8. Effects of Passing a Current through Cells.—(*a*) *Polarization of membranes* by continuous current. *Plant cells.* Use cells with an indicator pigment, for instance, epidermis of leaves of onion bulb or Coleus leaves. Pass a continuous current from a few dry cells in series through the tissue for a few minutes and observe under microscope. Do the cells become differently coloured at either end? If so note how the acid and alkaline reactions are placed with respect to cathode and anode. Explain.

**Frog Muscle.*—Connect two keys (*A* and *B*) in circuit with two dry cells. Connect a frog's muscle, by means of non-polarizable electrodes about one centimeter apart, with the binding posts of one of the keys (*A*). Close the circuit through the muscle for several minutes by means of key *B*, leaving key *A* open. Now open key *B* and immediately make and break key *A* several times. Does the muscle contract? Where is the source of the current in this experiment? The muscle acts as delicate galvanometer.

(*b*) *Permeability change* with alternating current (stimulation). Expose *Spirogyra* lying in acid fuchsin or cyanol to an alternating

(induction) current of intensity insufficient to injure (seriously at least). Does dye enter the cells? Does it enter in absence of the stimulation?

9. Electrosmosis and Electrophoresis (Reuss's experiment, 1808).

Two open tubes are partly immersed in wet clay and filled with water. A strong continuous current (from the main) is sent through by means of metal electrodes dipping into the tubes. Water rises in the cathode tube and falls in the anode tube (electrosmosis). The anode tube becomes cloudy with clay (electrophoresis).

10. Anomalous Osmosis.—This is probably related to electrosmosis. Set up an osmoscope with pig's bladder (preferably) or gut, inserting a capillary tube through a rubber stopper into the larger tube. Fill the tube with water and set in M/2 tartaric or citric acid (Flusin, 1908). Observe throughout the laboratory period and again next day.

Repeat with potassium citrate M/10 inside and isotonic to hypertonic (M 3 to M 2) sugar outside. Consider how the solution used affects:

1. The potential difference at the respective boundaries of the membrane.
2. Diffusion potential in the pores between the two solutions.
3. Electrokinetic potential on the walls of the pores.

Make a diagram for the citrate salt solution similar to that given for the acid on p. 136.

Outline X

COLLOIDS IN PHYSICAL SYSTEMS

I. SUSPENSIONS, EMULSIONS AND SUSPENSOIDS

Colloids are finely heterogeneous systems. Some of their properties are illustrated by systems of a coarser heterogeneity, namely, suspensions and emulsions, as in the first exercise below.

1. Effect of Particle Size and Proportion of Internal to External Phase on Viscosity and Consistency.— [(*a*) Note the physical properties of mixture of (a) sand, (b) clay, with water—(1) of the " suspension " when the two are shaken up and (2) of the sediment with excess water poured off.]

(*b*) Shake up a little oil with water to which an *emulsifying* agent, such as gum acacia, has been added. (The rôle of emulsifiers has already been studied.) Note variation in viscosity as the amount of oil is increased up to a point, after which the emulsion " breaks." Study the relation of phases at intervals. These may be distinguished by adding water-soluble methylene blue or oil-soluble Sudan III. How is viscosity related to the relative volume of the internal and external phase?

2. Optical Properties. (*a*) *Brownian Movement.*—This is visible under the ordinary microscope in the case of fine suspensions such as carmine but is better studied with dark field illumination. Note the relation of amplitude of vibration to size of particle and to viscosity of medium (add glycerine).

(*b*) *Faraday-Tyndall Effect.*—Note opalescence of fine suspensions and even homogeneous-looking colloid solutions when held in a strong beam of light. It is this same scattered light which is received into the microscope in ultramicroscopy. View the opalescence through a Nicol's prism. From the side with the prism axis at right angles to the beam there is extinction, showing that the scattered light is plane polarized at right angles to the path of the beam.

(*c*) *Ultramicroscopic Appearance.*—See Outline XII.

*3. **Electric Charge: Electrophoresis.** (See also Reuss's experiment, p. 233.)—*Apparatus:* A U-tube and platinum electrodes held in rubber corks for each arm of the tube. Nearly fill the tube with colloid solution. Run a layer of distilled water on top of each arm. Dip in the electrodes to about 2–3 mm. above the colloid solutions. Pass a current from the main for 20–30 minutes. Try carbon, congo red, etc.

4. Peptization (dispersion).—(*a*) Pour an *alcoholic solution of oil* into water. Why does the dispersed oil remain suspended?

(*b*) Disperse carbon in water by shaking. Do the same in a solution of ammonia. Allow tubes to stand. Compare stability.

5. Flocculation or Precipitation by Ions. (*a*) *Relation to Valency of Ion.*—Use the above carbon suspension or, better, diluted india ink or arsenious sulphide (see below) and test the relative precipitating power of various electrolytes. Does NaCl in any concentration serve to precipitate? Find the respective lowest concentrations = flocculation values (F.V.) of $BaCl_2$ and $LaCl_3$ which cause precipitation under equal conditions and in equal times as follows:

To a known quantity of sol in a test-tube add known small quantity of salt solution; shake and wait for signs of precipitation—first seen as carbon flocks adhering to sides of tube. Repeat with 1/10 of the former concentration of salt and so on diluting by tenths until no precipitate occurs on standing. What is the relation between precipitating power and valency? Does a salt with polyvalent anion ($K_4Fe(CN)_6$) have any effect? What charge do you infer to be borne by the carbon particles?

(*b*) *Relation to Adsorbability, etc., of the Ion.*—Using carbon as colloid determine the flocculating values of HCl, $AgNO_3$, and of a basic dye, like methylene blue. Any change in concentration of the dye in solution? Also add an amount of HCl insufficient to coagulate and compare the effect of NaCl with that in absence of acid.

*(*c*) *Flocculation Value.*[1]—Preparation of the As_2S_3 solution. Dissolve 3 grams of As_2O_3 in $\frac{1}{2}$ litre of distilled water by boiling the mixture in a steam cleansed Jena flask with reflux water-cooler.

[1] From Bartell's Class Outline of experiments in Colloid Chemistry. Experiments arranged by Kruyt.

Put 200 cc. distilled water into a flask and let H_2S gas pass through this liquid; then let the As_2O_3 solution run into the H_2S solution by drops, without interrupting the gas stream. Finally the surplus of H_2S is driven out by a stream of hydrogen (washed successively through flasks with $KMnO_4$, Na_2CO_3, $HgCl_2$, NaOH and water).

For the estimation of the limit value for KCl, $BaCl_2$ and $Al_2(SO_4)_3$, make standard solutions containing 300 milliatoms (0.3 N) K, 6 milliatoms (0.006 N) Ba, 1 milliatom (0.001 N) Al. Make dilutions from the standard solutions:

1 : 1 1 : 2 1 : 4 1 : 9 1 : 14

Bring into Kruyt flocculation-beakers (or test-tubes) 10 cc. of As_2S_3 solution and 5 cc. of electrolyte solution, shake them, leave them for two hours, shake again and state two minutes later between which concentrations the limit value lies. The limit value is expressed in milliatoms per liter (total volume sol + electroyte solution).

The limit value is (approximately) for K 70, for Ba 1, for Al 0.2 milliatom per liter.

Make the same investigation for new fuchsene (limit value 0.2 millimol) and pay attention to the special differences between the flocculation here and with the salts just mentioned. Repeptization in higher concentrations of new fuchsene.

6. Periodical Precipitation: Liesegang Rings.—Gelatin 3 per cent containing potassium dichromate 0.1 per cent is spread in a thin layer in a petri dish and allowed to set. Two drops of concentrated (50 per cent) $AgNO_3$ are placed some distance apart on the film. Cover and observe development of the rings in question.

The experiment may be varied by drawing the gelatin into a capillary tube and allowing the $AgNO_3$ to diffuse from the end. Other combinations of reacting salts may be tried.

II. EMULSOIDS

1. Diffusibility: Dialysis. Colloids and Crystalloids. (Thomas Graham, Philosoph. Trans. 1861.)—Using a small dialyzer (gut tied over the end of a flanged glass tube), dialyze a mixture of starch solution and NaCl. Test the dialysate for both substances, with iodine in KI and $AgNO_3$, respectively. Repeat with egg albumin as the colloid.

2. Dispersion in Relation to Electrolytes. (*a*) *Gelatin Sol.*—Does $LaCl_3$ (polyvalent cation) or $K_4Fe(CN)_6$ (polyvalent anion) cause coagulation? Try the effect of a saturated salt solution $(NH_4)_2SO_4$ — "salting out." Optical properties — any Tyndall effect? Ultramicroscopic visibility? Note high viscosity and gelation on cooling.

(*b*) *Albumin Sol.*—White of egg diluted with four times its volume of H_2O may be used. In some of its characters this appears intermediate between lyophilic and lyophobic colloids.

Coagulation by Electrolytes.—Note that only a trivalent cation ($La^{\cdots}$ N, 100) is able to cause precipitation and that much higher concentration is necessary than for suspensoids.

Amphoterism.—What is the reaction of egg white to neutral red or litmus? Acidify with acetic acid. If acid is gradually added an increase followed by a decrease of opalescence may be observed. Significance? Compare action of polyvalent cations ($LaCl_3$) and polyvalent anions ($K_4Fe(CN)_6$) with that on the natural albumin. Effect of acid on electric charge? Does neutralization of charge precipitate the albumin particles?

3. Protective Action of Emulsoids.—Mix a little warm gelatin solution with an equal quantity of $\frac{M}{10}$ $Pb(NO_3)_2$. (Some of the gelatin is precipitated, but not enough to prevent gelation.) Do the same with a starch solution. Place a drop of each on a slide; cover with a coverglass and allow to set. Cover also a drop of $Pb(NO_3)_2$ in water. Then place a drop of $\frac{M}{2}$ $K_2Cr_2O_7$ at the edge of each coverglass. Compare the precipitates when the dichromate diffuses in.

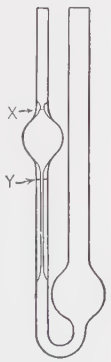

Fig. 66.

*4. **Electroviscous Effect (Kruyt) in Sols.**—*Special Apparatus:* Viscosimeter (Fig. 66); cleaning mixture; stop watch.

Prepare 1 per cent solution of soluble starch. To a paste of 5 grams soluble starch add 400 cc. boiling water. Shake and boil for fifteen minutes. When cool make up to 500 cc. Keep overnight at 25° C. Filter through ash-free filter.

Prepare N/250, KCl, $BaCl_2$, $LaCl_3$. Dilute portions of each, to obtain also N/500, N/1000 and N/2000—25 cc. of each concentration required. Make the following

mixtures and *measure viscosity* of each at 25° C. immediately on mixing:

To successive 25 cc. portions of starch solution add 25 cc. of the following: H_2O
KCl N/1000, N/500, N/250
BaCl N/2000, N/500, N/250
$LaCl_3$ N/2000, N/1000, N/500
H_2O

The first and last measurements allow interpolation of time change if any in the sol. so as to obtain true zero value. Bring results into a graph.

Measurement of Viscosity.—The viscosimeter must be thoroughly clean and dry. Pipette 5 cc. of liquid into the viscosimeter; suck up to the level of the top mark (*X*). Measure accurately with the watch the time taken for the liquid to fall from the top to the bottom mark (*Y*). The viscosity varies inversely as this time (see p. 162).

5. Lyotropic Influence of Salts on Gelation.—The following salt solutions are required: M 2 K_2SO_4, KCl and KCNS, the last made neutral by the addition of HCl. (If desired some students may use a neutral mixture of acid and basic phosphates, K acetate and KI brought to neutral *p*H, so as to extend the ion series.) Put 10 cc. of warm gelatin (say 6 per cent) into each of four vials and mix an equal volume of M 2 K_2SO_4, M 2 KCl, M 2 KCNS and distilled H_2O respectively with it. Note the time taken to set and the relative consistency of the respective gels.

6. Swelling of Gels.—Influence of electolytes.

Protein.—Prepare gelatin strips about 5 cm. × 1 cm. Set up ten wide petri dishes containing respectively:

(*a*) distilled water
(*b*) N/500, N/50 and N/5 HCl
(*c*) N/500, N/50 and N/5 KOH
(*d*) N/10 NaCl, N/10 NaCl + N/50 HCl, N/10 HaCl + N/50 KOH

Immerse a strip in each. Do not let them stick to the glass. Measure length at intervals during 1–2 hours or longer. (The *p*H of the media may be measured at the close.) Plot length of strip versus *p*H or acid concentration.

[*Carbohydrate.*—Equal amounts of granulated agar are placed in 4 test tubes of uniform bore to which are added respectively:

H_2O, N/20 HCl, N/20 KOH, N/10 NaCl.]

Outline XI

COLLOIDS IN THE CELL

1. Irreversible Coagulation of Protoplasm.—Study with the ultramicroscope the action of acid (H-ions), lanthanum or aluminium salt (trivalent ions) and heat in causing coagulation of protoplasm (see Outline XII).

2. Reversible Changes in Dispersion of Cell Colloids. (*a*) *Cytoplasm.—Root hairs* under ultramicroscope. Use acetic acid, say M 1000 and at first indication of increase in ultramicrons neutralize and see if the flocculation is reversed (see Outline XII).

(*b*) *Nucleus.*—Large cells of stigma of lily flowers, etc., may be used. Note reversible aggregation of chromatin particles with weak acetic acid and dispersion with weak ammonia.

(*c*) *Granules and Sap.*—(1) Agent—Highly absorbable organic ions (basic dye). Carefully observe the behaviour of cytoplasmic granules in *Spirogyra* when exposed to neutral red—to which some $CaCl_2$ has been added to reduce staining of the cell wall. Do the granules flocculate on staining? How might this be explained in view of the positive charge on the coloured ion of the dye? Does the staining substance (partly tannin) in the vacuole precipitate? Treat a portion of the preparation with an alkaloid (1 per cent caffeine). Behaviour on standing? (Compare effect of caffeine on an unstained preparation.) Treat cells with acetic acid (M/1000). Do the granules lose their colour or do they dispsese? Do they behave in these respects as an amphoteric colloid?

[(2) Agent—H-ions. Make sections of (dry) stem of *Guayule.* Note rubber emulsoid in the sap of certain cells. Ammonia stabilizes, acetic acid flocculates.]

3. Estimation of Protoplasmic Viscosity.—From the following observations and experiments try to obtain a conception of the physical state of the different parts of a *Spirogyra* protoplast, comparing also different species of *Spirogyra.* (Of course, other cells may be used as well.)

(*a*) Compare the vigour and amplitude of Brownian movement of particles in the cytoplasm, the cell sap and in the water outside. (See also Experiments with ultramicroscope.)

(*b*) *Note* rate of kinoplasmic streaming in the cytoplasm.

(*c*) *Note* plasmolysis shapes in the early stages of plasmolysis and the ultimate form of the contracted protoplast. (The plasmolyzing agent may be $\frac{M}{4}$ $CaCl_2$.) The time taken to round off into the "convex" plasmolysis form. Evidence of adhesion to the cell wall, of viscosity in the cytoplasm as a whole or of viscosity or rigidity of the chloroplasts as determining factors. Specific differences.

*(*d*) Centrifuge filaments of *Spirogyra* for varying lengths of time.

Note rate and degree of displacement of chloroplasts, granules, nucleus, etc. Compare different species.

*(*e*) A demonstration of micrurgy gives the student a particularly vivid idea of the viscosity and in many cases elasticity of protoplasm. The technique is easy with naked cells (amoebae, sea urchin eggs, etc.). Plant cells require a special technique in virtue of their tough cell walls. The Chambers, Janse-Peterfi, and Taylor types of micro-manipulator are now well known. They are worked by fine screws. A new *lever type* of manipulator of remarkable and complicated design, by J. Florian, Masaryk University, Brno, allows free hand movement of the needles in any direction.

4. Viscosity Change—Electroviscous (?) Effect in Protoplasm.—Note that $LaCl_3$ like $BaCl_2$ (already observed) produces reduction in surface of the chloroplasts, etc., of *Spirogyra*. Compare the lowest concentration effective in the two cases and the absence of result with dilute monovalent cations. Note also the effect of these ions on Brownian movement of cytoplasmic granules, that is, on viscosity. Consider reduction of electric charge on the surface of, and reduction of viscosity in, the chloroplasts as possible factors in accounting for their behaviour.

Treat *Spirogyra* with $AlCl_3$ and subsequently with a plasmolytic agent. Note evidence of adhesion of protoplasm to wall.

5. Swelling and Shrinkage of Cell Colloids.—(1) *In Relation to H-ion Concentration.*

(*a*) Turgor changes in stomatal movement. Make tangential

sections of the lower epidermis of *Tradescantia*. Allow guard cells to stain in dilute neutral red, preferably while kept in the dark. Sketch, indicating carefully the volume of stained sap colloid. Treat one preparation with M 500 NH_4OH and another with M 1000 acetic acid. Volume change of sap colloid and of the whole cell? Corresponding changes in stomatal aperture?

*(*b*) Expose roots with healthy root hairs to dilute acetate buffers ranging from *p*H 5 to *p*H 7. Observe any variations in thickness of layer of cytoplasm in the root hairs, also in rate of streaming and number of visible granules. (Two swelling minima about *p*H 6.4 and *p*H 5.9 have been recorded with an intermediate maximum about 6.1.) Do hairs burst in any of the solutions?

(2) *In Relation to Salts of Alkalis.*—Plasmolyze cells of red onion epidermis with M 3 KCl and M 3 NaCl, and follow subsequent changes in particular cells in respective solutions. Does the plasma layer thicken as time elapses (observe after one or two hours)? Does it become more hyaline? Any difference between NaCl and KCl?

***6. Growth in Relation to Swelling.**—Among the factors influencing growth is turgor, which in turn is partly determined by swelling pressure and by the *p*H of the cells. The rate of growth of root hairs in the above solutions, or of sweet pea pollen in various dilutions of acetic acid and ammonia (+ cane sugar) may be studied as a possible illustration. In all the above experiments, however, especially those dealing with growth, the factors involved are complex and it is by no means certain that the simplest possible explanation is the true or complete one. Assuming that it is, what are the conclusions as to an isoelectric point or isoelectric points for protoplasm?

7. Protoplasmic Contraction.— Restudy amoeboid movement, looking for viscosity changes and gel contraction as a feature of the mechanism. Focus carefully on the upper surface of an amoeba in locomotion and then on the deeper, forward streaming layer and compare Brownian movement of granules in each. Does the outer region (ectoplasm) appear to be fluid or gelatinous. If the latter, consider the significance of the narrowing of the ectoplasmic tube posteriorly.

Restudy from the same standpoint *Spirogyra* when treated with M 100 $BaCl_2$. Note that the chloroplasts may shorten

(condense their spiral) without becoming smooth and rounded, as if still gelatinous in consistency.

Restudy *Paramoecium* as regards *ciliary movement.* Mount in suspension of china ink. Can the cilia reverse their effective beat. Treat with NH_4OH, N/1000. Note changes undergone by cilia and vacuoles. If swelling occurs, note if cilia still beat when the surface is lifted off the body. *Cryptochilon*, found in the alimentary tract of the sea urchin, is a particularly good subject for study of ciliary movement.

Outline XII

MICROSCOPY WITH DARK-FIELD ILLUMINATION

(The so-called ultramicroscopy)

1. Technique.—Dark-field microscopy, known since ca. 1850 (Ross, Wenham, Stevenson) became practically important about 1900. Although not generally used as yet except in restricted fields, the method is of increasing importance in all biological branches of microscopy, and is particularly important in medicine, bacteriology, protobiology generally, and in the study of plant and animal protoplasm, secretions, excretions, etc.

The purpose of the present outline is to guide the student in the use of the dark-field condenser, the optical arrangement which makes " ultramicroscopy " possible. By this means objects too minute to be seen with the ordinary condenser (light-field) are brought into view; but in addition optical effects are produced which assist toward a more exhaustive interpretation of objects ordinarily visible with the microscope.

The method consists in directing a very brilliant beam of light laterally or obliquely upon the objects to be studied. The form of this beam is such as to preclude it entering the objective front lens; or, if it does so, it enters the marginal zone of the lens only, and then must be excluded from vision by a suitable stop placed within the objective. The beam is a hollow cone, at the apex of which the object is illuminated. This hollow cone may be upright (u.l.c.) or inverted by reflection from the coverglass (i.l.c.). In the former case, the light in part traverses the object (if transparent) before reaching the eye, supplementing light reflected from surfaces. Diffraction images are also produced, the form of which is a guide to the form of the object if too small to be plainly visible. In the latter case with dark-field illumination only diffracted and fluorescent light reaches the eye.

Illumination: Light from a 400-watt condensed filament lamp or 4-amp. arc, or other small but powerful source of light, condensed

into a narrow parallel beam directed at the center of the flat mirror of the microscope. The mirror has to be arranged so that the beam emerges symmetrically as a hollow cone from the upper face of the d-f-condenser. This may be tested by means of a piece of uranium glass. *Oil* (nujol) or *glycerine is always used to make optical contact with condenser.* This applies to slide as well as uranium glass.

Object slides should be about 1 mm. for "upright light cone," 0.8 mm. or less for "inverted light cone," varying somewhat according to the make of condenser. A bit of wetted tissue paper on the slide will help in centering. This is done by screws projecting from lower edge of condenser mounting. Center first under 16 mm. objective, and then 4 mm.

2. Examination of Physical Colloids and Body Fluids. (1) *Suspensoids.*—Mount a drop of a suspension of carbon in water (india ink) and place for examination (oil contact). With 16-mm. objective observe the change in form of light image on raising or lowering the condenser: low—circle; intermediate, when focal point lies in the film of fluid—a spot; high—a circle, with inner fainter circle or spot because the apex of the cone is now reflected downward (inverted light cone). When spot is first visible in the field one is using the upright light cone.

Observe the carbon suspension using u.l.c. and 4-mm. objective followed by 2 mm., making contact between cover slip and lens with immersion oil. Note: brilliancy of particles; sizes (uniform or otherwise?); Brownian movement (amplitude in relation to size of particles); differences in object pictures when using upright light cone and inverted light cone as to brilliancy and extent of diffraction images, noting the form of these images carefully, and their appearance in relation to the focal plane of the objective.

Relation of Brownian Movement to Viscosity of Medium.—Examine a suitable suspension, such as gamboge: (*a*) in water and (*b*) in strong glycerine, and compare amplitude of movement of the two.

Examine various sols, and note any difference in appearance: *silver, gamboge, mastic.*

(2) *Emulsoids.*—Is anything visible in a gelatin sol?

(3) *Body Fluids,* Secretions or Excretions. *Latices: Latex containing caoutchouc,* obtainable from *Ficus elastica* or *Hevea.*

It is necessary to dilute and stabilize with ammonia. Note the different kinds of suspensoids resp. emulsoids. The larger of caoutchouc; the smaller of proteins, etc. *Coagulation:* On adding acetic acid at margin of cover slip flocculation of the protein suspensoids and enmeshing of the caoutchouc emulsoids may be observed.

Milk: Cow's milk diluted, the cream removed as far as possible. Numerous casein suspensoids with fewer large butter-fat droplets. Flocculation of casein follows on adding acid to edge of coverslip.

3. Examination of Cells. (*a*) *Cell Suspensions. Blood.*—A small drop of blood is mounted as usual. The form of the *erythrocytes* may be easily studied. Small particles of *chyle* (fat) in rapid Brownian movement. In open spaces the origin and accumulation of *fibrin* filaments can be seen. They commonly start from foci where *blood platelets* have stuck to the glass and disintegrated. Observe these. Here and there find *leucocytes* appearing as a cloud of white granules, the whole somewhat larger than a red cell. The heat of the lamp often induces them to amoeboid movement. The granules spring locally into Brownian movement as the viscosity is lowered preparatory to the extrusion of a pseudopod.

Saliva.—Squamous epithelial cells; motile bacteria; curious globules with inclosed particles in active Brownian movement.

Suitable cultures of *Bacteria*, motile and non-motile; in some forms the flagella can be seen. Character of the movements?

(*b*) *Cell Contents.*—Diffraction and reflection from the cell wall reduce the light entering the cell and sometimes dazzle the eye so that, altogether, invisibility of internal structures when the wall is bright is not proof of optical homogeneity. A mount in glycerine will indicate whether scattering by the wall is concealing the contents. Of course the cell will be plasmolyzed or will collapse.

Spirogyra.—Cytoplasm almost optically empty, chloroplasts slightly cloudy, pyrenoids and starch conspicuous. Suspensoids and sometimes crystals in the vacuole in Brownian movement. Try the effect of heat, alcohol, $BaCl_2$, acetic acid: clouding of the cytoplasm and chloroplasts—coagulation. See also (*c*) below.

Root hairs (as those of a grass seedling germinated on blotting paper). After observing normal root hairs, treat with acetic acid N/1000 and at first sign of appearance of ultramicrons (smallest

visible particles) neutralize with weak ammonia. Is the coagulation reversed?

Paramoecium.—Study structure and ciliary movement and coagulation of protoplasm.

(*c*) *Fluorescent Pigment in Cells. Blue-green algae.*—With u.l.c. these are various shades of blue-green and red-green. With i.l.c. they are red fluorescent or orange fluorescent respectively. In water, the occurrence and distribution of bright granules may be made out; in glycerol the reflecting surfaces are obviated and a fuller degree of fluorescence is seen. The heat developed at the light apex may cause loss of fluorescent pigment which leaching out into the surrounding medium affords a bright fluorescent spot of light.

Green Algae: Spirogyra.—Mounted in glycerol, the red fluorescence of the chloroplast may perhaps be seen. If a suitable light filter is interposed between the illuminant and the object, the fluorescence can be more easily seen. The filter should cut out the red and orange.

Other green algae may be examined for fluorescence with various degrees of success. In some of the diatoms it is readily observable.

Red algae: The fresh-water *Chantransia* affords a mixture of red and orange fluorescence.

Outline XIII

GENERAL PROPERTIES OF ENZYMES

These experiments are such as illustrate the properties of enzymes in relation to their colloidal nature and their resemblances to living matter. The classification of enzymes is a subject for biochemistry. In the following experiments involving digestion of starch either commercial diastase (a plant enzyme) or saliva may be used.

1. The Process of Digestion of Starch (*a*) *Erosion of Starch Grains.*—Prepare sections or a quantity of scrapings of wheat endosperm; take two watchglasses *A* and *B*, with two other watchglasses to serve as covers. To *A*, add some of the scrapings or sections and a little diastase solution, to *B*, add water only. Cover the watchglasses to check evaporation, and put them in a moderately warm place (temperature 20°–35° C.). After several hours, mount the sections or the isolated grains of the scrapings in a drop of water, examine them microscopically and observe that the grains which have been acted on by the diastase begin to show signs of change; they swell, radial fissures appear, each grain comes to have a corroded appearance, and subsequently nothing remains of it but a pale outline or ghost. Apply the iodine test to the grains in various stages of dissolution.

Compare the appearance of starch in green and ripe bananas.

(*b*) *Stages in Digestion.*—Make a starch paste by adding boiling water to a thick paste of starch made with cold water. Stir, allow to settle, and decant from the sediment. Collect saliva, dilute with 4 volumes of water and filter. Place about 12 cc. saliva preparation in 20 cc. starch paste. Test at frequent intervals for starch and glucose by removing samples and treating one with I in KI, heating another with Fehling's solutions. The pinkish reaction with iodine before complete hydrolysis indicates erythrodextrin, an intermediate product. Note the gradual

increase of transparency as the colloidal solution becomes crystalloidal.

(*c*) *Specificity of Enzyme Action.*—Expose other raw foodstuffs than starch to the action of saliva or diastase and test for digestive products, for instance, cane sugar (test for glucose); milk (coagulation?), etc.

2. Factors Regulating Enzyme Activity. (*a*) [1] *Temperature.*—Influence of temperature on enzyme action: quantitative determination. Collect saliva, dilute with 7 volumes of water and filter. Add 5 cc. dilute starch paste to each of four test-tubes. Place one test-tube in the water bath at 40° C., and keep another at room temperature (about 20° C.). Have two series of iodine drops ready on a porcelain plate. When the test-tubes have attained the proper temperatures add an equal volume of dilute saliva to each tube, mix thoroughly, and keep at their respective temperatures. At intervals of one-half minute remove a drop of the mixture and test for starch. One student may attend to the test from 40° saliva mixture, another to the 20° mixture. Determine the time required to convert the starch into products giving no colour reaction with iodine at each temperature.

Perform exactly the same experiment, using the *same* diluted saliva, but keep one test-tube at 0° C. and compare it with another at 20° C. Record your results in the from of a table. How near in agreement are the two determinations at 20° C.? What is the increase in rate of enzyme action for a rise of 20° C.? Heat saliva to 80° C. and test its activity.

Enzymes, like protoplasm, show an *optimum temperature.* This simply means that the natural increase of chemical activity with rise of temperature is interrupted by injury to the enzyme itself.

(*b*) *Poisons.*—Inactivation may be brought about by many chemical agents which affect the colloidal state of the enzyme-agents and which also act as poisons to protoplasm. Test the activity saliva or diatase after treatment with $HgNO_3$, alcohol, tannin, etc.

(*c*) *H-ion Concentration.*—Compare the rate of salivary action on starch in buffer solutions of varied *p*H, for example, phosphates at *p*H 6, 7 and 8, and other buffers in a wider range. Compare diastase. Is the optimum *p*H the same? According to Michaelis,

[1] This experiment and no. 3 below follow Harvey (Laboratory Directions in General Physiology, Princeton).

enzymes are amphoteric and most active at their isoelectric point.

3. Relation of Enzyme Activity to Concentration of Enzyme and Substrate. (*a*) *Concentration of Enzyme.*—Collect saliva and filter. Add 5 cc. dilute starch paste to each of two test-tubes. Have a series of iodine drops ready on a porcelain plate as in experiment 2 above. Add as follows to the two test-tubes:

(a) 5 cc. of 1 part saliva to 3 parts water;
(b) 5 cc. of 1 part saliva to 7 parts water.

Keep at 20° C. and determine by trials at one-minute intervals when the iodine gives no colour reaction with the mixture.

After making the first series of experiments perform the same experiments using 5 cc. starch paste and the same saliva diluted as follows:

(c) 5 cc. of 1 part saliva to 15 parts H_2O.
(d) 5 cc. of 1 part saliva to 31 parts H_2O.

Keep at 20° C. and determine by trials at ten-minute intervals the time required to convert the starch to a substance giving no colour with iodine.

*(*b*) *Concentration of Substrate.*—Reversibility of some enzyme action. If the chemical reaction concerned (respectively decomposition or synthesis) is reversible, depending on the concentration of the reagents, it may be accelerated in both ways by the same enzyme. Lipase is the most favourable enzyme to demonstrate this. It occurs in plants but is most easily obtained from the pancreas of animals. The following reaction is involved in the experiment given:

$$\text{Ester} + \text{water} \rightleftharpoons \text{acid} + \text{alcohol}$$

$$\text{Ethyl butyrate} + H_2O \rightleftharpoons \text{butyric acid} + \text{ethyl alcohol}$$

Hydrolysis.—Equal amounts of ethyl butyrate are placed in two flasks. To one some fresh pancreatic extract or commercial pancreatin is added; to another boiled extract in like amount. An acid range indicator, such as methyl red, is added to each. Cork tightly and keep at 40°. How does the reaction change? Relative rate in the two flasks? (The amount of acid produced after 40 minutes may be determined by titration, first cooling to 0° to stop the reaction.)

Synthesis.— Mix 25 cc. M/20 butyric acid and 10 cc. 20 per cent ethyl alcohol. Divide into two flasks and add equal quantities of fresh and boiled pancreatic extract as before, as well as a suitable indicator. Cork and keep at 40°. Compare rate of change of reaction. After 40 minutes compare the odor of the two flasks. Is ethyl butyrate detectable in either? It has an odour of pineapples.

From the above experiments note points of resemblance of enzymes to physical colloids on one hand and to protoplasm on the other.

APPENDIX

APPARATUS AND MATERIALS

I. Student's Individual Outfit.—Microscope; 1 alcohol lamp (if gas is not available); 1 tumbler; 2 glass preparation dishes with lids; 5 large shell vials with corks; 5 small shell vials with corks; 6 test tubes; 1 10-c.c. graduated cylinder; 2 osmometer outfits, that is, open glass tubes (2″ or 3″ long) with perforated rubber corks holding pieces of capillary tube and clothes-pin as holder; 2 watchglasses; 1 (or more) petri dish; 2 medicine droppers; 2 needles, mounted; 1 small forceps; microscope slides and coverglasses; 1 celluloid millimeter scale. Extra equipment required for certain experiments is noted in the list of materials below.

II. Biological Material.

Outline I: Snowberry (fruits), *Spirogyra Elodea, Tradescantia* (flowers), *Vaucheria, Nitella, Paramoecium.*

Outline II: *Fern* with ripe sporangia.

Outline III: *Pollen, Vaucheria, Spirogyra spp., Paramoecium, Elodea, Tradescantia, Amoeba.*

Outline V: Blood, *Spirogyra,* red onion, green and ripe bananas, plant stem, leaves (*Anthericum* or *Vicia*) female cockroach, *Amoeba.*

Outline VII: *Spirogyra, Coleus* or other cells suitable for plasmolysis; blood, frog, protozoa, *Anthericum* or *Vicia, Coleus, Symphytum* (flowers), rhubarb or celery stalks, *Tradescantia* leaves.

Outline VIII: *Paramoecium, Tradescantia,* yeast, etc.

Outline IX: Apple, frog, red onion, *Spirogyra.*

Outline XI: *Spirogyra, Tradescantia,* root hairs, *Lilium* (flowers), red onion, *Paramoecium.*

Outline XII: Latex from *Hevea* or *Ficus*, etc., milk, blood, saliva, bacteria, blue green and red algae, *Spirogya*, *Paramoecium*, root hairs.

Outline XIII: Wheat grains, green and ripe bananas, fresh pancreas or pancreatin.

III. Reagents and Extra Equipment. Distilled water, methyl alcohol and ethyl alcohol always set out.

Outline I: Cane sugar M/4, neutral red, Janus green, china ink.

Outline II:

I. Needles, copper wire, fine thread, parts of surface tensiometer, dry cells with wires. Soap, salt, nujol, mercury, dichromate crystals, solid paraffin, 50 per cent glycerine, lecithin.

II. Glass filaments. Camphor, mercury, HNO_3 (1N), olive oil, chloroform, Sudan III, soda crystals, carbon, red ink or eosin, shellac (chloroform soluble), chloroform, egg yolk.

Outline III: Dry cell and induction coil with wires and glass tubing. KNO_3 M/3, $BaCl_2$ M/100, chloroform solution.

Outline IV:

I. Tensiometer. Solution of bile salt (Na taurocholate or glycocholate) 1 part in 500, methylene blue, soap, saponin, egg-white, nujol, olive oil, chloroform, xylol, lamp-black, powdered $BaSO_4$ or $CaCO_3$, M/10 $CaCl_2$, M/10 NaOH, animal charcoal, benzene.

*Adsorption isotherm experiment. Activated charcoal, oxalic acid 1N, KOH 0.1N, 5 glass-stoppered bottles, 10 cc. pipette, burette.

II. Filter paper. Methylene blue, indigo carmine, basic fuchsin, acid fuchsin, eosinate of methylene blue, methyl orange or bromphenol blue (slightly acidified), neutral red or bromthymol blue, dilute HCl, NaCl N/1, $AlCl_3$ N/1000, gelatin.

Outline V:

I. Saponin solution, 1.8 per cent NaCl, eosin, alcoholic iodine solution, KNO_3 1N, ethyl alcohol 11 per cent, butyl aclohol $1\frac{1}{4}$ per cent, acetone, $K_4Fe(CN)_6$ ca. N/900, $FeCl_3$ M/100, rennet solution, carbon, milk, tannic acid, caffeine 1 per cent, dilute NaOH.

II. Methylene blue, neutral red, methyl orange, dilute HCl, dilute NaOH (M/100) NH_4OH (M/100), 0.7 per cent NaCl.

OUTLINE VI:

I. Four small bottles for each student, cotton, agar (ca. $1\frac{1}{2}$ per cent), brom cresol green, neutral red, gelatin sol., 0.5 M HCl, H_2SO_4 and citric acid, $CuSO_4$ 10 per cent, crystals of $K_4Fe(CN)_6$, $CuSO_4$ and $CoCl_2$, fused $CaCl_2$, solutions of $CuSO_4$ (3 per cent), $K_4Fe(CN)_6$, sodium silicate, Na_2CO_3 (concentrated), tannic acid (2 per cent), cane sugar M/2, 20 per cent gelatin + $K_4Cr_2O_7$, $K_4Fe(CN)_6$ 2.1 grams. per liter, also 0.31 gram per liter, $CuSO_4$ 2.5 grams per liter, also 0.74 gram per liter, cane sugar 1M, cane sugar (unknown), KCl, $AgNO_3$, aniline blue, lecithin in xylol, NH_4OH M/100, NaOH M/500.

[Demonstration: glass cylinder, phenol, saturated solution of $CuSO_4$.]

Animal charcoal, methylene blue, nujol, phenol.

OUTLINE VII: M 2 cane sugar, glucose, glycerol, urea, alcohol, NaCl and $CaCl_2$; sugar (unknown) caffeine 1 per cent, urea 1 per cent, NH_4OH M/100, NaOH M/200, acetic acid M/1000, HCl M/1000, 1 per cent orange G. eosin, congo red and methylene blue, ether 5 per cent, 0.28 M NaCl, $BaCl_2$ M/100; M/10 $CaCl_2$, $SrCl_2$ $ZnCl_2$, YCl_3 and $AlCl_3$; $LaCl_3$ M/100.

OUTLINE VIII:

(*a*) *Indicator Method.*—Buffer solutions or colour standards.

Indicators:	Per Cent
Thymol blue (T. B.)	0.04
Brom phenol blue (B. P. B.)	0.04
Brom cresol green (B. C. G.)	0.04
Methyl red (M. R.)	0.04
Chlor phenol red (C. P. R.)	0.04
Brom cresol purple (B. C. P.)	0.04
Brown thymol blue (B. T. B.)	0.04
Neutral red (N. R.)	0.04
Phenol red (P. R.)	0.02
Cresol red (C. R.)	0.02
Meta cresol purple (M. C. P.)	0.04
Cresol phthalein (C. P.)	0.04

0.2N HCl, gelatin, NHCl M/10, NH_4OH M/1000, NaOH M/2000, soil.

(*b*) **Potentiometric Method.*—Potentiometer, 2 bright platinum (or gold) electrodes with mercury tubes fitted in rubber stoppers, wires, 2 vials, KCl bridge, standard acetate solution, quinhydrone crystals. (Wheatstone bridge, 2 dry cells, standard Weston cell, galvanometer.)

Outline IX: **Measurement of Potential.*—Potentiometer, 2 calomel electrodes (capillary electrometer for experiments, with frog muscle), 2 open tubes, clay, pig's bladder, dry cells. M/10 NaCl, KCl, $MgCl_2$, K_2SO_4 and KNO_3.

Acid fuchsin, citric acid M/2, K citrate M/10, cane sugar M/2.

Outline X: Sand, clay, nujol, gum acacia, methylene blue, Sudan III (powder), carmine suspension, glycerine, (U-tube, platinum electrodes). Dry carbon, diluted india ink, congo red, ammonia solution, NaCl M/1, $BaCl_2$ M/10, $LaCl_3$ M/100, HCl M/100, $AgNO_3$ M/100, methylene blue M/100.

*Adsorption isotherm experiment: As_2S_3 sol. KCl 0.3N, $BaCl_2$ 0.006N, $AlCl_3$ 0.001N, new fuchsin 0.001N.

Gelatin 3 per cent $K_2Cr_2O_7$ 0.01 per cent, $AgNO_3$ 50 per cent, NaCl starch solution, I in KI, diluted $AgNO_3$. $K_4Fe(CN)_6$ M/10, gelatin sol, albumin sol, acetic acid.

* Electroviscous effect experiment: Viscosimeter, starch solution N/250, KCl, $BaCl_2$ and $LaCl_3$.

Six per cent gelatin, M/2 K_2SO_4, KCl and KCNS.

Outline XI: Acetic acid M/1000, NH_4OH M/500, neutral red, $CaCl_2$ M/20, 1 per cent caffeine, $BaCl_2$ M/100, $LaCl_3$ M/100, N/1 NaOH, N/1 acetic acid, M/3 KCl, M/3 NaCl, china ink.

Outline XII: Dark field condenser, lamp providing powerful condensed source of light, bull's-eye condenser lens, uranium glass, tissue paper, nujol, glycerine.

Colloids: Such as diluted india ink, gamboge, silver, mastic, gelatin. Latex of *Hevea* or *Ficus*, strong acetic acid, milk, acetic acid M/1000.

Outline XIII: Thermometer, ice, starch, diastase, I in KI, cane sugar, dilute $HgNO_3$, tannin, phosphate and other buffers. *Ethyl butyrate, butyric acid M/20, pancreatic extract.

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